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A HERITABLE ABNORMALITY IN THE GERMINATION OF CHLAMYDOSPORES OF USTILAGO ZEAE[1]

ST. JOHN P. CHILTON

(Accepted for publication January 12, 1943)

INTRODUCTION

In the course of studies on the genetics of *Ustilago zeae* (Beckm.) Unger, chlamydospores from certain crosses between haploid cultures were found to germinate abnormally. Certain monosporidial cultures, capable of producing galls and chlamydospores when inoculated singly into corn plants, and, therefore, designated as solopathogenic lines, were found among the progeny of these abnormally germinating chlamydospores. A brief report of these studies was made previously (1), and this paper gives a more detailed account of the results obtained.

REVIEW OF LITERATURE

The general literature concerning the genetics and cytology of *Ustilago zeae* has been reviewed (2, 3, 9, 17), and only certain details need be repeated here. Abnormal germination of smut chlamydospores has been reported by Christensen (3) in one cross in *U. zeae*, by Goldschmidt (7) in *U. violacea*, by Hanna and Popp (10) and Holton (11) in interspecific crosses between *U. avenae* and *U. levis*, by Rodenhiser (14) in crosses between *Sphacelotheca sorghi* and *S. cruenta*, by Laskaris (13) in *S. sorghi*, and by Kienholz and Heald (12) in *Tilletia tritici*. Christensen (3) and Holton (11) suggested that a lethal factor or series of factors might account for the peculiarities in germination that they observed. Laskaris (13) showed that some type of segregation occurred for normal and abnormal germination. Goldschmidt (7) interpreted his results as indicating cytoplasmic inheritance.

Solopathogenic lines in *U. zeae* have been reported by several investigators (1, 2, 3, 5, 18). Christensen (3) found that such lines were indistinguishable in culture from haploid ones, were not dicaryotic, remained solopathogenic for varying lengths of time in culture, and, on losing solopathogenicity, changed in cultural characters also. Sporidia from chlamydospores produced by these lines were generally haploid, although certain chlamydospores again produced solopathogenic lines.

MATERIALS AND METHODS

The source and parentage of the cultures of *Ustilago zeae* used in the present studies are given in table 1. All cultures, except 15 from cross 50, were derived from single sporidia isolated with a glass needle in a micro-

[1] Summary of a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy, granted by the University of Minnesota, July, 1938.

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manipulator, using the technique of Dickinson (4) and Hanna (8). The 15 cultures from cross 50 grew from hyphal tips cut from the promycelia.

TABLE 1.—*Origin of cultures used, their source, parentage, and type of isolation*

Cross number or identification letters and numbers	Parentage	Type of isolation	Where obtained
10	A2 by A3	Complete sets[a]	E. C. Stakman
11	10A2 × 10H3	" "	"
12	10K4 × 10G4	" "	"
14	10K2–1 × 10I1	" "	"
15	10K4 × 10F2	" "	"
16	10A4 × 10C1	" "	"
17	10K2–1 × 10I1	" "	"
18	10K2 × 10I1	" "	"
21	16B2 × 16B3	" "	"
22	14G3 × 14I3	" "	"
23	15E2 × 15E4	" "	"
25	14B1 × 14B3	" "	"
26	15E3 × 15E4	" "	"
33	22B2 × 22B4	" "	"
40	10A4 × 15C2	Random[b]	Writer
41	40a × 40i	"	"
42	10A4 × 15C1	"	"
44	10A4 × U.F. A3	"	"
45	U.F. A1 × A4	"	"
46	16B2 × U.F. A4	"	"
47	Solopathogenic line 40n–1	Complete sets	"
48	21H1 × U.F. A4	" "	"
50	10K2–1 × 18H1	Complete sets + 15 Hyphal tip cultures	M. F. Kernkamp
51	Solopathogenic line 40n	Complete sets	Writer
53	46B3 × U.F. A4	" "	"
56	10K4 × 40i	Random	"
100	10K2–1 × 14B3–3	Complete sets	E. C. Stakman
101	100H4 × 202A4	" "	"
102	100C4 × 202A4	" "	"
202	22B4 × 33A2	" "	"
300	14J4 × 26G1–2	" "	"
301	17B4 × 26G1–2	" "	"
U.F.	Chlamydospores from University Farm, St. Paul, Minn.	" "	Writer
Fargo	Chlamydospores from Fargo, N. D.	" "	"

[a] Cultures from all 4 sporidia from a promycelium were secured wherever possible.
[b] No attempt made to secure cultures from all primary sporidia from a promycelium.

The system used to identify the various cultures was as follows: An arbitrary number was assigned to each cross and to chlamydospores produced by solopathogenic lines. Letters or words were used to designate the source of the material when isolations were made from material collected in the field. Where sporidia were isolated from a promycelium and the position of each was known, the chlamydospore was identified by a capital letter and the sporidia were numbered from 1 to 4, progressing from the distal to the proximal cell. For example, 16A1 designates a line obtained from the sporidium isolated from the distal cell of the promycelium of chlamydospore

A from Cross 16. If sporidia were isolated at random from promycelia, the cultures were designated by one or more small letters placed after the cross number (40a or 40aa). Genetic variants (mutants) are indicated by a number placed after the culture designation and separated by a dash (16A1–1 or 40a–1).

In the experiments on sex and pathogenicity, Northwestern Dent and Picaninny corn plants were grown in sterilized soil in 4- or 6-inch pots from seed treated with an organic dust. The inoculation technique described by Stakman and Christensen (16) was followed. Solopathogenicity tests were made by inoculating plants with monosporidial cultures. Except where stated, a minimum of two tests, with 10 to 20 plants in each test, was made with each cross or solopathogenic line; and in many cases 8 to 10 tests were made.

Cultures were grown on 50 cc. or 30 cc. of potato-dextrose agar (10 g. dextrose, 15 g. agar, and infusion of 300 g. of potatoes to a liter of medium) in 250-cc. or 125-cc. Erlenmeyer flasks. All the medium used in each experiment was made at the same time, and the flasks were sterilized in the autoclave in groups of 100, the sterilization time and pressure being alike for the groups. Duplicate flasks were inoculated with each culture and were incubated at room temperature for 5 to 8 weeks, after which notes were taken.

The studies on chlamydospore germination were made at room temperature on thin films of potato-dextrose agar on sterile cover slips inverted on sterile Van Tieghem cells in Petri dishes. Moistened filter paper was used in the bottom of the dishes. Special media are described in connection with the experiments in which they were used. Chlamydospores were spread on the agar films with a glass needle in a micromanipulator, preparatory to isolating single sporidia.

GERMINATION OF CHLAMYDOSPORES

Chlamydospores that germinate normally produce a promycelium with 3 or 4 cells on each of which sporidia are usually formed (Fig. 1, A). The chlamydospore may act as the fourth cell in some cases, producing one or more sporidia.

The chlamydospores that germinated abnormally produced promycelia in about the same length of time as those that germinated normally, but some of the promycelia collapsed and the contents exuded before septa were formed (Fig. 1, B). The word lysis will be used hereafter to designate this disintegration. A round body suggestive of an abortive sporidium was often produced on the side of the promycelium (Fig. 1, D). Lysis occurred if the chlamydospores were dusted on agar films, indicating it was not due to rough handling. Promycelia that did not lyse generally produced viable sporidia that grew into colonies, although in some cases the isolated sporidia budded 2 or 3 times and then died. This limited budding was seen occasionally also in otherwise normally germinating collections of chlamydospores. Many of the promycelia producing sporidia were gnarled and twisted (Fig. 1, C), others were made up of large, irregular cells, and it was difficult to find pro-

mycelia with 4 cells in any of the abnormal material studied. Long hyphal branches sometimes were formed directly from the chlamydospores or from the promycelia. A few germination types superficially similar to those found in the Tilletiaceae were seen, a whorl of sporidia or sporidia and

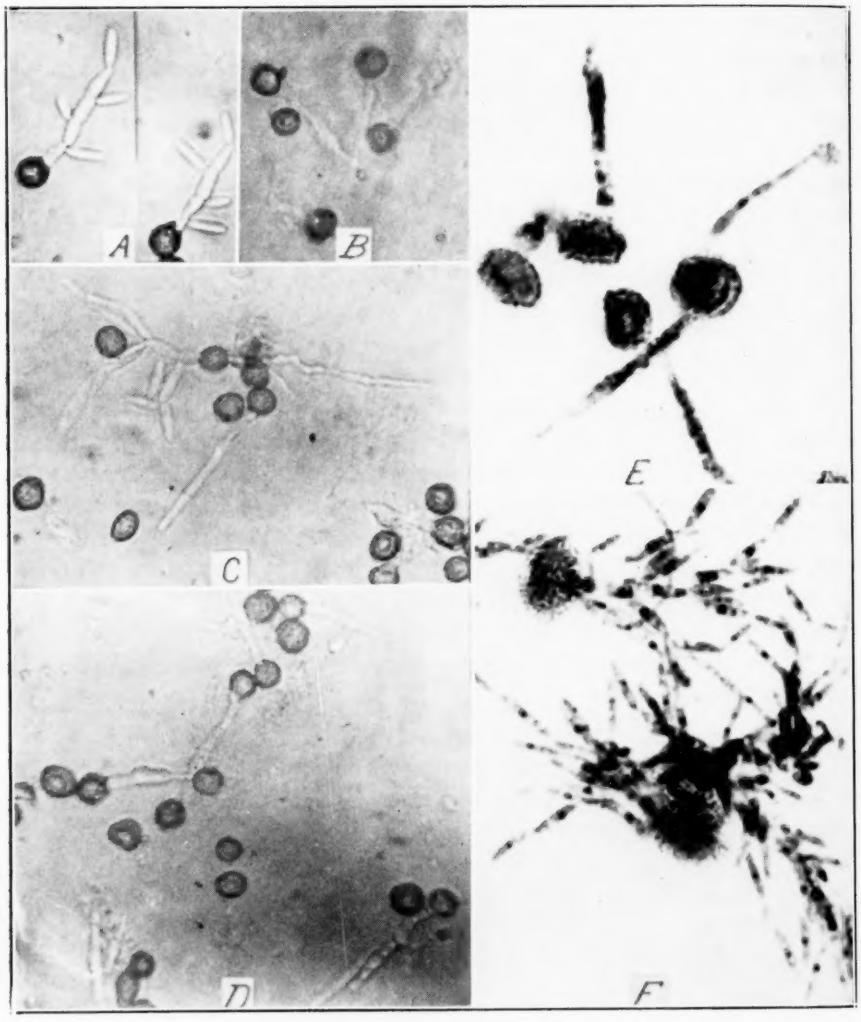

FIG. 1. Photomicrographs of abnormally and normally germinating chlamydospores. A. Normally germinating chlamydospores. B–D. Abnormally germinating chlamydospores. E. Abnormally germinating chlamydospores stained with hematoxylin. F. Normally germinating chlamydospores stained with hematoxylin.

hyphal branches being formed at the tip of a one-cell promycelium. Many chlamydospores produced 2 irregular promycelia.

The proportion of promycelia that lysed varied from 76 to 95 per cent in the various crosses studied. In one case a consistent 15 per cent difference in the number of promycelia that lysed was found between two col-

lections of chlamydospores produced by inoculations made at different times with the same two compatible lines.

When enough promycelia were examined from crosses that produced otherwise normally germinating chlamydospores, a few promycelia were seen to lyse in nearly every case, the percentage varying from 8.3 to 0.3 per cent. This occurred, even when the chlamydospores were dusted on coverslips to eliminate the possibility of injury.

Normally (Fig. 1, F) and abnormally germinating chlamydospores (Fig. 1, E) on the same films of agar were stained with hematoxylin in accordance with the technique described by Hanna (9). The cytoplasm in many of the abnormal promycelia stained much darker than that of the normal ones on the same cover slip; and there often was a concentration of cytoplasm in various parts of a promycelium (Fig. 1, E). Nuclei were observed in chlamydospores prior to germination, at the beginning of germination, in the promycelia producing sporidia, and in the sporidia themselves.

INFLUENCE OF ENVIRONMENT ON ABNORMAL GERMINATION

Several collections of chlamydospores that had germinated normally and abnormally, respectively, in previous tests on potato-dextrose agar were tested in sterile distilled water; in potato broth with 1 per cent and 20 per cent dextrose; in 2 per cent water agar, with and without 0.1 per cent NaCl; and on corn-meal agar, malt agar, potato-dextrose agar, and on potato-dextrose agar to which 1, 5, and 10 drops of N/10 NaOH, and 1, 2, 3, and 5 drops of commercial lactic acid, respectively, had been added to 10-cc. portions. None of these media was responsible for the peculiarities of germination of the chlamydospores of the various collections. Lysis occurred in the abnormally germinating chlamydospores at 15, 20, 25, and 30 degrees C.

Attempts to Induce Abnormal Germination

On the assumption that lysis might be due to a virus or some similar substance, a few chlamydospores were placed on a thin film of agar and left until promycelia were produced and lysed. Chlamydospores from a normally germinating collection were then added but there was no lysis or other observable abnormality, even in promycelia in direct contact with the lysed ones. This was repeated many times, with the same negative results.

Small pellets of agar on which 5 to 6 chlamydospores had germinated and the promycelia had lysed were added to compatible lines growing in potato dextrose broth, and these were left standing for approximately a week. The lines were then mixed, inoculated into corn plants, and the chlamydospores that were produced germinated normally.

These results indicate that the abnormality was not due to an infectious agent, or at least was not transmitted by the methods used.

Segregation for Abnormal and Normal Germination

The chlamydospores produced by the original cross 10, from which most of the material used in these studies was obtained, germinated abnormally.

Haploid lines isolated from this cross were mated in various compatible combinations, and some of the resultant crosses produced chlamydospores that germinated abnormally, which others produced chlamydospores that

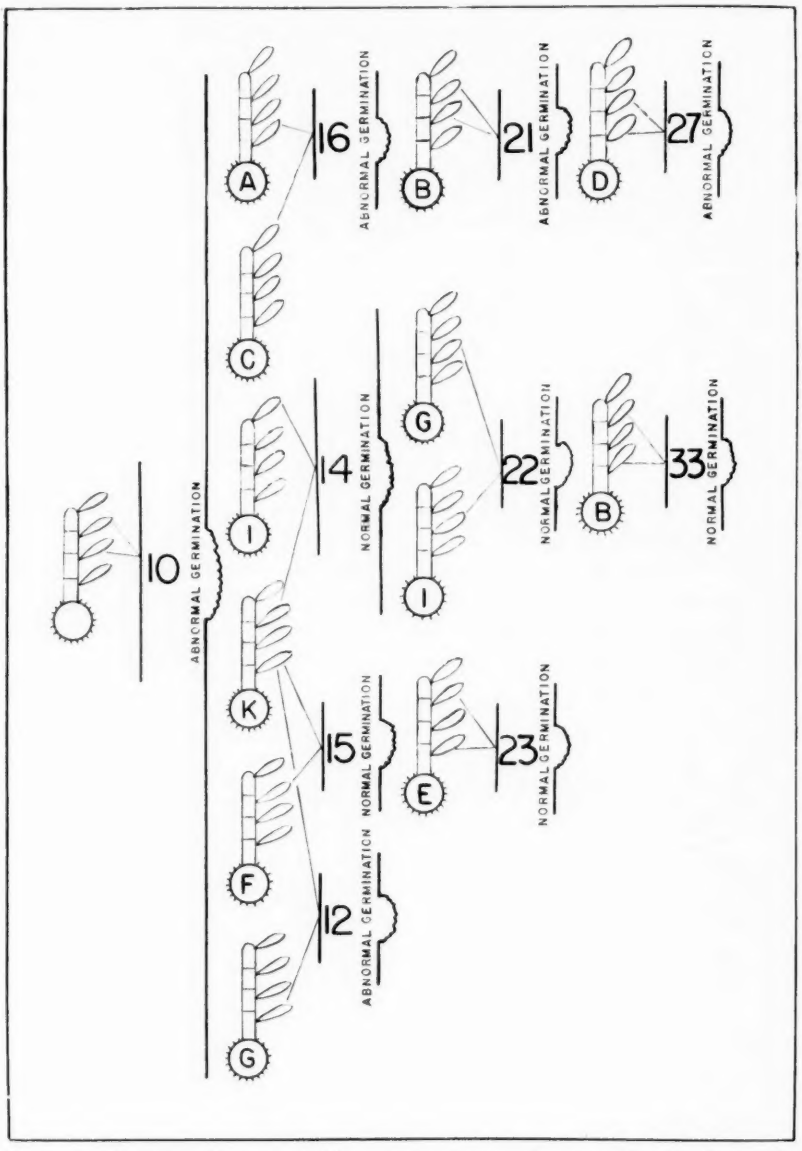

FIG. 2. Diagrammatic presentation of segregation for normal and abnormal germination of chlamydospores of *Ustilago zeae*.

germinated normally. Monosporidial isolates from these crosses were intercrossed in turn. The results, presented diagrammatically in figure 2, show that chlamydospore lines were obtained from the original cross in which normal germination occurred for 3 and 2 successive chlamydospore gener-

ations, respectively, and that other chlamydospore lines were obtained in which abnormal germination occurred for 3 and 1 successive chlamydospore generations, respectively. A segregation of some type occurred for abnormal and normal germination of chlamydospores.

From 5 of the crosses made within the progeny of the original cross 10 (Fig. 2) there were 13 haploid lines which produced abnormally germinat-

TABLE 2.—*Results of crosses*[a] *made to determine whether there was segregation for normal and abnormal germination*[b] *of chlamydospores*

Lines crossed	Fargo B1	Fargo B2	10K2	10K4	10H3	10C1	10I1	U.F. A4	15C1	15C2–1	16B2	21D3	21H1	40a
10A2					A									
10A4						A				A				
10C1	N[b]													
10C2	N													
10F1		N												
10F2	N			N										
10G4				A										
10H4	N													
10I1			N											
10I3	N													
10K2		N					N							
10K4		N					N							
12D4		A												
12E1		N												
16A1								N						
16A4		N						N						
16B2								N						
16B3	A[b]										A			
21A4		N												
21C3		N												
21D3	N													
21D4												A		
21F2	N													
21H1		N						N						
21H2		N						N						
21H3		A						A						
21H4	N												N	
21I4	N													
40a		N												
40f										A				
40i				A						A				A
40q									A	A				
40r–1									A	A				
40dd										A				
40B3		A												

[a] Crosses (as diagrammed in Fig. 2): 10 = A2 × A3
12 = 10K4 × 10G4
16 = 10A4 × 10C1
21 = 16B2 × 16B3
40 = 10A4 × 15C2

[b] N indicates normal germination; A indicates abnormal germination.

ing chlamydospores and 23 lines which produced normally germinating chlamydospores when mated with various tester lines (Table 2). The tester lines all produced normally germinating chlamydospores when mated among themselves.

From each of two crosses that produced normally germinating chlamydospores a haploid line was secured which, when crossed with tester lines, produced chlamydospores that germinated abnormally.

OCCURRENCE OF SOLOPATHOGENIC LINES

Sporidia were isolated from collections of normally and abnormally germinating chlamydospores produced by various crosses, and the cultures obtained were tested for solopathogenicity by inoculation tests. It may be seen from the data in table 3 that 31 or 19.6 per cent of the 158 abnormally

TABLE 3.—*Occurrence of solopathogenic lines among progeny of 29 crosses*

Cross number	Parentage	Occurrence of abnormal germination	No. chlamydospores from which one or more isolates were tested	Number isolates tested	No. chlamydospores from which solopathogenic lines were isolated	No. of solopathogenic lines
10	A2 × A3	+	11	39	3	11
11	10A2 × 10H3	+	8	24	0	0
16	10A4 × 10C1	+	6	16	0	0
42	10A4 × 15C1	+	24	24	3	3
40	10A4 × 15C2	+	39	39	10	10
44	10A4 × U.F. A3	+	6	20	1	4
12	10G4 × 10K4	+	7	20	2	2
21	16B2 × 16B3	+	6	16	0	0
41	40a × 40i	+	31	40[a]	6	6
56	40i × 10K4	+	1	1	1	1
45	U.F. A1 × A4	+	19	19	5	5
	Total		158	258	31	42
14 and 17	10K2–1 × 10I1	–	20	54	0	0
18	10K2 × 10I1	–	8	24	0	0
50	10K2–1 × 18H1	–	22	39	0	0
100	10K2–1 × 14B3–3	–	4	8	0	0
15	10K4 × 10F2	–	5	22	0	0
25	14B1 × 14B3	–	5	13	0	0
22	14G3 × 14I3	–	4	16	0	0
23	15E2 × 15E4	–	5	20	1	1[b]
26	15E3 × 15E4	–	7	10	0	0
46	16B2 × U.F. A4	–	18	39	0	0
300	14J4 × 26G1–2	–	7	13	0	0
301	17B4 × 26G1–2	–	7	9	0	0
48	21H1 × U.F. A4	–	11	23	0	0
33	22B2 × 22B4	–	5	19	0	0
202	22B4 × 33A2	–	8	20	0	0
53	46B3 × U.F. A4	–	4	7	0	0
101	100H4 × 202A4	–	1	2	0	0
102	100C4 × 202A4	–	4	4	0	0
	Total		145	342	1	1

[a] Some of the haploid lines from this cross were tested alone only once. The solopathogenic lines, however, were tested several times.

[b] This line's cultural characters changed and solopathogenicity was lost on transfer of stock culture.

germinating chlamydospores from which one or more cultures were isolated and tested, gave rise to one or more solopathogenic lines. There were 42 solopathogenic lines among the 258 monosporidial isolates tested, an average of 16.3 per cent. Three hundred and forty two lines from 145 chlamydospores from 18 crosses producing normally germinating chlamydospores were tested and only one line, or 0.29 per cent, was solopathogenic. This line, however, lost its solopathogenicity on transfer of the stock culture.

Five solopathogenic lines were found among 19 monosporidial isolates from the abnormally germinating chlamydospores produced by cross 45 (U.F. A1 × U.F. A4). This indicates that solopathogenic lines were not restricted to the relatively closely related lines used in these studies, as the chlamydospores from which cultures U.F. A1 and A4 were secured were collected in the experimental plots at University Farm, St. Paul, Minnesota.

Cultural Characters of Solopathogenic Lines

From 5 crosses, cultures of lines that had been proved by inoculation to be solopathogenic were compared with each other and their parents. The results (Table 4) show that solopathogenic lines from a given cross may be

TABLE 4.—*Comparison of cultural characters of solopathogenic lines with each other and with parental types*

Cross number	Parental lines	No. of solopathogenic lines compared	Parent resembled	No. of cultural types
40	10A4 × 15C2	10	10A4	7
41	40a × 40i	6	40i	3
42	10A4 × 15C1	3	10A4	2
44	10A4 × U.F. A3	4	Both	1
45	U.F. A1 × U.F. A4	5	Neither	5

the same or different, culturally. No character was found by which these lines could be distinguished culturally from haploid ones. These results are in accord with those of Christensen (2, 3).

Stability of Solopathogenic Lines

Lines were tested for solopathogenicity at various intervals, and the results (Table 5) show that some lines remained solopathogenic after 4 years

TABLE 5.—*Results of testing 31 originally solopathogenic lines after varying lengths of time in culture*

Cross from which lines were obtained	Age in months when last tested	No. of lines tested	Number solopathogenic	Number not solopathogenic
10	48	8	7	1
12	36	1	1	0
40	13	9	9	0
40	9	1	1	0
41	11	5	5	0
42	8	4	4	0
44	5	4	1	3

in culture, while others lost this character after a few months. Where solopathogenicity was lost, the cultural characters changed also.

Single sporidia were isolated from solopathogenic lines at various times and the cultures obtained were tested by inoculation into corn plants. The results (Table 6) show that monosporidial isolates from such lines may or may not be solopathogenic.

TABLE 6.—*Results of inoculations with sub-monosporidial isolates from solopathogenic lines*

Solopathogenic lines	Number sub-monosporidial isolates tested	Number solopathogenic	Number not solopathogenic
10J4	7	5	2
40n	2	1	1
40m	1	1	0
41a–1	1	1	0
41g	1	1	0
41v	1	1	0
41k	1	1	0
41gg	1	1	0
41kk	1	1	0
42r	1	1	0
42u	1	1	0
44A1	2	0	2
44A2	1	0	1
44A3	1	0	1
44A4	1	0	1
45g	1	1	0
45l	1	0	1
45n	1	1	0
45q	1	1	0

Monosporidial isolates were made from variants arising in solopathogenic lines and were inoculated into corn plants. Of 14 monosporidial isolates made from 5 variants, all were solopathogenic. These results agree also with those obtained by Christensen (3).

The non-solopathogenic monosporidial isolate from the solopathogenic line 44A3 (Table 6) was inoculated into corn plants in combination with each of 2 haploid compatible lines. It formed galls and chlamydospores in combination with one line and not with the other. This test was not repeated, and too much emphasis should not be placed on it. It suggests, however, that a delayed segregation for compatibility factors may possibly account for loss of solopathogenicity in culture.

Germination of Chlamydospores Produced by Solopathogenic Lines

In some cases chlamydospores formed by solopathogenic lines produced fewer promycelia that lysed than the chlamydospores from the original cross from which these lines were obtained. To eliminate the possibility that excessive handling of the chlamydospores in spreading them on the agar might cause lysis, chlamydospores produced by 6 solopathogenic lines were placed on thin films of sterile agar and touched lightly with other films of agar to spread the spores. Some of the promycelia still lysed.

Forty-two to 96 per cent of the chlamydospores from 10 solopathogenic lines from 3 crosses produced promycelia that lysed (Table 7). From

TABLE 7.—*Comparison of percentage promycelial lysis in chlamydospores produced by three lethal crosses and by solopathogenic lines from these crosses*

Source of chlamydospores	No. spores germinated	Percentage of lysed promycelia
Cross 40 (10A4 × 15C2)	653	85.0
Six solopathogenic lines from Cross 40	1853	42.1
Cross 44 (10A4 × U.F. A3)	265	80.4
Two solopathogenic lines from Cross 44	287	47.7
Cross 45 (U.F. A1 × U.F. A4)	867	94.2
Two solopathogenic lines from Cross 45	543	96.3

these data it may be seen that the chlamydospores obtained from the solopathogenic lines from crosses 40 (10A4 × 15C2) and 44 (10A4 × U.F. A3) produced fewer promycelia that lysed than did the chlamydospores from the original crosses. The 2 solopathogenic lines tested from cross 45 (U.F. A1 × U.F. A4) produced chlamydospores in which lysis occurred as often as in the original chlamydospore material from which they were obtained. Chlamydospores from 2 solopathogenic lines from cross 42 (10A4 × 15C1) were tested several times also, although no counts were made; it was found that fewer promycelia disintegrated than in the case of chlamydospores from the original cross.

Among chlamydospores produced by the solopathogenic lines from crosses 40, 42, and 44, no torulose and large-celled promycelia were observed. Most of these lines produced chlamydospores with promycelia that were apparently normal in shape; there was little difficulty in finding 4-celled promycelia with sporidia on each cell. In a few of these lines the chlamydospores formed promycelia with less than 4 cells.

Solopathogenic lines from the same cross differed with respect to the viability of the sporidia isolated. Single sporidia isolated from each of the cells of the promycelia of chlamydospores produced by solopathogenic lines 40n and 40n–1 grew readily, but a rather large number of the sporidia isolated from the promycelia of the chlamydospores produced by 40cc, 40e, and 40e–1 died or budded only once or twice. In some cases, the isolated sporidium produced a hyphal strand, which soon died. Sporidia were isolated from several collections obtained from inoculations made at different times and they behaved in the same way.

Differences in the percentage of promycelial lysis were found among chlamydospores produced by solopathogenic lines from the same cross. For example, 16.5 per cent of the promycelia from line 40e disintegrated, while 49 per cent and 50 per cent of those from lines 40n and 40n–1, respectively, disintegrated.

The germination of chlamydospores produced by 25 solopathogenic lines from 5 different crosses was observed. With the exception of the chlamydospores from the solopathogenic lines obtained from cross 45 (U.F. A1 ×

U.F. A4), the germination was much closer to normal than in the case of the chlamydospores produced by the original cross.

Segregation and Mutation in Solopathogenic Lines

From cross 40 (10A4 × 15C2) a solopathogenic line was obtained that was almost identical culturally to the 10A4 parent. A variant arose in this line, and a monosporidial isolate was made from it that proved to be solopathogenic. The variant later gave rise in turn to a variant, and a monosporidial isolate from it was also solopathogenic. At the same time it was found that the original 15C2 parent had produced a variant in culture which was still haploid, based on inoculation tests alone and in combination with compatible lines.

Culture 10A4 was brown and smooth on the surface. The culture 15C2 was brown also, but its surface was convoluted and the margin had counterclockwise lines. Culture 15C2–1, the variant from 15C2, was light buff and had a fine white mycelial covering over a rough surface. The variant 40n–1 from the solopathogenic line 40n was brown with a fine white mycelial growth over the surface also.

From the promycelia of 8 chlamydospores produced by the solopathogenic line 40n, 5 sets of 4 sporidia and 3 sets of 3 sporidia were isolated, and the resulting cultures were grown in duplicate flasks. Seven lines were obtained with cultural characters like 10A4 and 4 like 15C2. The other 16 lines were similar to both 10A4 and 15C2. Both the 10A4 and 15C2 types were recovered from the promycelia of 3 of the 8 chlamydospores.

From the chlamydospores produced by the solopathogenic variant 40n–1, 9 sets of 4 sporidia and 5 sets of 3 sporidia were isolated and compared in duplicate flasks. Five of these 51 lines were like 10A4 culturally, 5 resembled 15C2–1 rather closely, and 8 lines had a white overgrowth. Twenty other lines were like 10A4 in color but like 15C2–1 in topography, being rough on the surface. None was like 15C2.

Four of the monosporidial isolates from the chlamydospores produced by 40n and 16 of those obtained from 40n–1 were inoculated into corn plants alone and in various combinations. None was solopathogenic and all formed chlamydospores in certain combinations. These tests, repeated, gave similar results. Among the lines used were those resembling 10A4, 15C2, and 15C2–1 in cultural characteristics.

These results indicate that the solopathogenic line 40n was diploid, as the original parental types were recovered; and segregation occurred for sex or compatibility factors and for cultural characters. They also indicate that the variant from the solopathogenic line was produced as a result of a heritable change, and that rather similar mutations for cultural characters could occur in a genom whether alone or included in a nucleus with another genom.[2]

[2] Unpublished cytological results of E. C. Stakman have shown that the solopathogenic lines used in these studies were not dikaryotic.

CHLAMYDOSPORE SIZE

The chlamydospores that germinated abnormally were larger than those that germinated normally. Christensen (3) found this to be true also of the abnormally germinating chlamydospores he studied.

One hundred chlamydospores from each of 21 crosses were measured with an ocular screw micrometer, the spores being measured as they lay on a film of agar (Table 8). The data were analyzed by the method given by Fisher

TABLE 8.—*A comparison of average lengths and widths (in microns) of 100 chlamydospores from each of 21 crosses within Ustilago zeae*

Abnormally germinating chlamydospores			Normally germinating chlamydospores		
Cross	Length	Width	Cross	Length	Width
10A4 × 10C1	10.28	9.68	16A1 × U.F. A4	7.87	7.42
10A4 × 15C2	10.48	9.84	21H1 × U.F. A4	8.39	8.05
16A3 × U.F. A1	10.57	8.06	21H2 × U.F. A4	8.35	7.74
16B3–1 × Fargo B1	11.05	9.46	21H4 × Fargo B1	8.29	7.89
21H3 × U.F. A4	10.82	9.01	21C3 × Fargo B2	8.49	7.57
21H3 × Fargo B2	10.83	9.92	22B2 × Fargo B2	8.39	7.91
21A1 × U.F. A1	9.65	9.07	46D1 × U.F. A4	8.89	7.24
40f × 15C2	11.03	9.37	36E3 × 16B2	8.86	7.27
40a × 40i	10.16	9.56	Fargo B1 × B2	8.57	8.13
44B3 × Fargo B2	10.72	9.80	U.F. A4 × Fargo B1	8.63	8.05
U.F. A1 × A4	11.69	8.14			

(11), and the "F" value was obtained from the tables of Snedecor (15). The lengths and widths of the abnormally germinating chlamydospores produced by the 11 lethal crosses were 9.7 μ – 11.7 μ by 8.1 μ – 9.9 μ; and 7.9 μ – 8.9 μ by 7.2 μ – 8.1 μ in the case of the normally germinating chlamydospores

TABLE 9.—*Analysis of variance of lengths and widths of chlamydospores of 21 crosses of Ustilago zeae*

Variation	Degrees of freedom	Sum of squares	Variance	F
Length				
Within abnormally germinating group	10	3,111.51	311.15	8.04[b]
Within normally germinating group	9	1,446.50	160.72	4.16[a]
Between two groups	1	48,097.99	48,097.99	1,243.48[b]
Within crosses	2079	80,409.08	38.68	
Total	2099	133,065.08		
Width				
Within abnormally germinating group	10	7,416.46	741.46	40.19[b]
Within normally germinating group	9	1.784.44	198.27	10.74[b]
Between two groups	1	22,050.55	22,050.55	1,194.5[b]
Within crosses	2079	38,383.55	18.46	
Total	2099	69,635.00		

[a] Value of "F" exceeds 5 per cent point.
[b] Value of "F" greatly exceeds 1 per cent point.

from the 10 non-lethal crosses. The chlamydospores from many of the lethal crosses were rather irregular in outline, and a few were found with distinct apicula.

Chlamydospores produced by 90 crosses among the various lines used in these studies were checked by observation as to size and then were germinated. The chlamydospores of 28 of these crosses were large and germinated abnormally, while those produced by the other 62 crosses were smaller and germinated in the normal way.

The data in table 9 not only indicate that the difference in size is significant between chlamydospores produced by lethal and non-lethal crosses, but also show that there were significant differences among the various collections measured.

Chlamydospores produced by solopathogenic lines were compared with those of chlamydospores from the original crosses from which the solopathogenic lines arose. Chlamydospores produced by two crosses between compatible haploid lines obtained from the chlamydospores produced by the solopathogenic line 40n–1 were measured also. The data (Table 10) show

TABLE 10.—*Comparative widths and lengths of chlamydospores of 2 abnormally germinating crosses, 6 solopathogenic lines, and 2 crosses between segregates from solopathogenic line 40n–1.*

Source of chlamydospores	Length (in microns)	Width (in microns)
Cross 40 (10A4 × 15C2)	10.48	9.84
Solopathogenic line 40e	8.51	7.69
Solopathogenic line 40m	8.28	7.60
Solopathogenic line 40n	8.45	7.93
Solopathogenic line 40n–1	8.28	7.60
47A1 × A2[a]	7.95	7.76
47D2 × D3[a]	8.46	7.81
Cross 45 (U.F. A1 × A4)	11.69	8.14
Solopathogenic line 45p	10.48	8.34
Solopathogenic line 45q	11.71	8.44

[a] Cross made between 2 segregates obtained by isolating sporidia from chlamydospores produced by solopathogenic line 40n–1.

that the chlamydospores produced by the solopathogenic lines obtained from cross 40 (10A4 × 15C2) were of the same size as those produced by crosses between non-lethal lines, as given in table 8. The chlamydospores produced by the solopathogenic lines from cross 45 (U.F. A1 × U.F. A4) were like those of the parental cross. Those produced by the two crosses between haploid isolates from 40n–1 were more nearly the size of the chlamydospores produced by the solopathogenic line 40n–1 than the ones produced by the original cross 40 (10A4 × 15C2).

As stated previously, the chlamydospores produced by the solopathogenic lines from crosses 40 (10A4 × 15C2) and 44 (10A4 × U.F. A3) were more nearly normal in their germination type than the chlamydospores of the original crosses from which they were obtained. In cross 45 (U.F. A1 × A4), however, the chlamydospores produced by the solopathogenic lines 45p and 45q germinated as abnormally as the original chlamydospores from which

they were obtained. Comparisons of germination type, percentage of lysis, and spore size are given in table 11. There is a correlation between spore

TABLE 11.—*Comparison of chlamydospore size, germination type, and lysis of original crosses, solopathogenic lines, and re-combinations of isolates from solopathogenic line 40n–1.*

Cross	Size[a] of chlamydospores	Type of germination	Percentage of lysis
40 (10A4 × 15C2)	Large	Abnormal	85.0
6 solopathogenic lines	Normal	Normal	42.1
47A1 × A2[b]	"	"	59.6
47D2 × D3	"	"	29.8
44 (10A4 × U.F. A3)	Large	Abnormal	80.4
2 solopathogenic lines	Normal	Normal	47.7
45 (U.F. A1 × A4)	Large	Abnormal	94.2
2 solopathogenic lines	"	"	96.3

[a] Size was determined by measurement or was estimated by visual comparison with other measured chlamydospores from similar lines.

[b] Haploid lines isolated from promycelia of chlamydospores produced by the solopathogenic line 40n–1.

size and abnormalities in germination, the solopathogenic lines from crosses 40 (10A4 × 15C2) and 44 (10A4 × U.F. A3) producing the smaller chlamydospores with the more normal germination, while the chlamydospores produced by the solopathogenic lines 45p and 45q were like those of the parental cross 45 (U.F. A1 × A4) with respect to size and abnormal germination.

DISCUSSION

Preceding experiments show that the abnormal germination of chlamydospores from certain crosses between haploid lines of *Ustilago zeae* is due to heritable factors. Haploid lines that carry the factor or factors for lysis, and diploid solopathogenic lines, can both be propagated indefinitely in culture; hence the operation of the lethal seems to be confined to the time in the life cycle when meiosis occurs. The high percentage of solopathogenic lines among the surviving monosporidial isolates from abnormally germinating chlamydospores indicates that the peculiar germination tends to be associated with a failure of normal disjunction of chromosomes. As the percentage of chlamydospores that lysed differed among chlamydospores produced by different crosses, it would seem that the abnormal germination may be controlled by multiple factors. The partial return to normal germination of chlamydospores produced by solopathogenic lines makes it difficult to account for the lethal on this basis. Chromosomal abnormalities preventing the normal disjunction of the chromosomes at meiosis, yet capable of a partial return to normal in the process of the reductional divisions, might possibly be the cause. This would account for the differences found among solopathogenic lines with respect to abnormal germination, their partial recovery, and the perpetuation of this recovery in the chlamydospores produced by crosses between compatible segregates from solopathogenic lines.

The correlation that occurs between abnormal germination and the increased size of the chlamydospores is unexplainable with the data available. It is interesting that Laskaris (13) found the same correlation in *Sphacelotheca sorghi*.

SUMMARY

Chlamydospores from certain crosses between monosporidial lines of *Ustilago zeae* germinate abnormally, producing gnarled and distorted promycelia that either autolyze before producing sporidia or produce relatively few sporidia in an irregular manner.

Extensive experiments indicate that the lysis is not caused by an infectious agent of the nature of bacteriophage.

The factor or factors for lysis are carried in certain lines only and segregation for factors affecting this character has been demonstrated by an appropriate series of crosses.

As the lysis is restricted to the promycelium, the operation of factors for lysis and other abnormalities associated with it seems to be restricted to the period during which meiosis occurs.

There was a definite tendency for the production of unusually large numbers of solopathogenic (apparently diploid) sporidia by promycelia of chlamydospores resulting from crosses involving one or more haploid lines carrying the factors for lysis.

Solopathogenic lines grew normally, caused infection when inoculated singly into corn plants, and in other respects behaved like similar lines described by other investigators.

Mutation occurs in solopathogenic lines, as well as in haploid lines.

There was partial reversion to the normal type of germination in the chlamydospores produced by some solopathogenic lines, as indicated by a decided decrease in the percentage of abnormal and disintegrating promycelia. When haploid sporidia, produced by such "reverting" lines were crossed, it was evident that the recovery persisted.

There is a correlation between the abnormally large size of chlamydospores and the tendency for these to germinate abnormally. This is unexplained, but, according to Laskaris, occurs in *Sphacelotheca sorghi*, also.

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EVIDENCE FOR THE EVOLUTION OF PHYTOPATHOGENIC VIRUSES FRO MMITOCHONDRIA AND THEIR DERIVATIVES.[1] II. CHEMICAL EVIDENCE

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INTRODUCTION

Following the presentation of cytological and genetic evidence for the evolution of viruses from plastids or mitochondria, this paper presents chemical evidence for such evolution by demonstrating the occurrence of a ribose nucleoprotein in the plant plastid, of the same general type that constitutes viruses. There exist already indications of the presence of nucleoproteins outside the nucleus, especially in the plastids.

1. Plastids either derive from chondriocontes, specialized mitochondria or proplastids, which divide, or by division of plastids already present (10).

2. Variegation-inducing plastids, producing symptoms like viruses, show heredity of their abnormal characteristics. Banga (1) and Bawden (2) indicated the presence of an extranuclear hereditary substance in the plastid: a nucleoprotein.

3. Claude (3) demonstrated the presence of nucleoprotein in particles of protoplasmic centrifugates of animal origin, presumably chondriosomes or mitochondria.

4. Reed and Dufrenoy (13), Banga and Szent-Gyorgyi (1) demonstrated the presence of phosphorus in the plastid (although lecithins as phosphorus carriers might have been involved here).

There exist also some indications of a relation between these nucleoproteins and virus nucleoproteins.

1. Bawden *et al.* (2) were the first to prove the presence of ribose nucleic acid in virus-proteins. Previous experiments have demonstrated the interrelation between tobacco-mosaic virus and the chromoprotein complex (20).

2. They also have demonstrated the dependence of both chromoprotein and virus-protein on the same building stones and the same respiration system (21).

EXPERIMENTAL

I. Isolation of Chloroplasts in Suspension

In order to separate the chromoprotein complex from the nuclei and cytoplasmic proteins, the preparation of pure plastids was necessary. For the first steps the method of Granick (9) was used with modifications.

Method. 100 g. of leaves of *Nicotiana tabacum* var. Maryland commercial were ground for 2 minutes in a Waring blender in an 11 per cent sucrose – .5 per cent thiourea solution. (The behavior of plastids in solu-

[1] Scientific paper No. A43, Contribution No. 1870 of the Maryland Agricultural Experiment Station (Department of Botany).

tions from 2–22 per cent was studied. A 5 per cent sucrose solution, generally considered isotonic with the cell, preserves the plastids poorly. Admixtures of salts tend to break up the plastids.) The suspension was kept at about 10° C. throughout the experiment. It was filtered through cheese cloth and then through pyrex glass wool. (Cytoplasmic strands occur when the preparation is not strained through glass wool.) The suspension was then subjected to selective centrifugation. Each time the pellet and the supernatant were examined microscopically.

Three minutes of centrifugation with an Adams Senior Angle Centrifuge

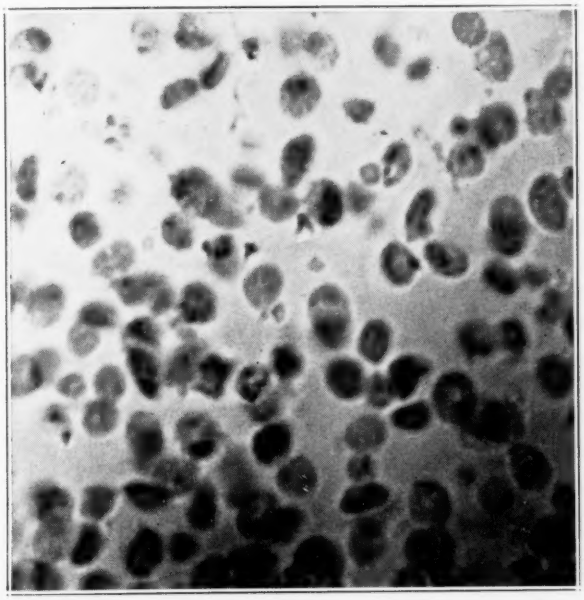

Fig. 1. Photomicrograph of purified plastid preparation of leaves of *Nicotiana tabacum* var. Turkish.

at about 1500 r.p.m. followed by 20 seconds at 3600 r.p.m. for packing threw down heavy débris, starch, plastids with starch, a great amount of triangular crystals, and nuclei. (Granick reports that nuclei disintegrate. Fortunately, in our material the nuclei were thrown down intact before the plastids.) The supernatant was centrifuged at about 4000 r.p.m. The pellet contained whole and some broken chloroplasts, and approximately 1 nucleus to each 1000 chloroplasts. The resuspended pellet was recentrifuged at low speed (1500 r.p.m.) and the new pellet discarded. The supernatant was recentrifuged at 4000 r.p.m. The pellet now contained mainly intact plastids (Fig. 1) with some plastid débris. The pellet was rewashed and recentrifuged. Preparations of this type are free of nuclei and débris and, if kept in the refrigerator at about 4° C., remain intact for months.

II. Extraction of the Nucleoprotein

Preliminary extractions of nucleoprotein were made from preparations of the chromoprotein complex of the whole cell content, since the loss of material through purification of plastids amounts to 60–80 per cent.

A. *Extraction from Extracts of the Whole Cell.* The general method used for the preparation of the chromoprotein complex has already been described (20). However, some important modifications were made.

(1) The material was ground in a Waring blender in 0.1 per cent Na_2HPO_4 buffer and 0.5 per cent thiourea. *Thiourea has proved an excellent anti-oxidant.* Denny (5) was the first to use it to prevent the oxidation of phenolic compounds.

(2) The ammonium sulphate, distilled water, and other solvents used were all brought to pH 7–8 in order to avoid any great deviation from the pH range of cytoplasm. In certain cases dilute sodium dodecylsulphate (0.1 to 0.05 per cent) was used where it was desirable to break up particles. Its use will be specifically mentioned.

For the determination of nucleoprotein, the chromoprotein preparation made according to the previously described method (20) was precipitated by adding an equal volume of 30 per cent $(NH_4)_2SO_4$, collected on a Johns-Manville hyflo supercel celite filter, was washed twice with 15, 7.5, 5 and 2.5 per cent $(NH_4)_2SO_4$. The filtercake was then washed with distilled water, followed by elution with .1 per cent Na_2HPO_4. This procedure was repeated, and the chromoprotein finally eluted with distilled water, brought to pH 8. Twice the volume of acetone was added in order to remove the pigments. The precipitated protein mixture was reextracted repeatedly with acetone. With the third or fourth wash the carotenoids came off, since these were more tightly bound than the chlorophylls. The preparation was then resuspended in chloroform-ether to remove lipoids, washed with alcohol, and finally with distilled water.

Digestion of the whole chromoprotein complex was accomplished either by 0.5 N NaOH for 3 hours at room temperature or 18 hours at 0° C., or by 0.1 N KOH for 24 hours at 0° C. The preparation was then brought to pH 8 and centrifuged. The pellet was re-extracted with either NaOH or KOH and the two supernatants added.

The liberated nucleoprotein was precipitated by bringing the solution to pH 4.8 with dilute HCl, followed by centrifugation. The nucleoprotein pellet was repeatedly washed with water of pH 4.8.

The nucleic acid was obtained from this nucleoprotein preparation by several methods.

(1) By dissolving the nucleoprotein in a NaOH solution at pH 8 followed by the addition of 5 times the volume of glacial acetic acid and centrifugation of the nucleic acid. The pellet was washed successively in 1 per cent HCl, alcohol, ether, then dried and tested.

(2) By dissolving the nucleoprotein in NaOH at pH 8, adjusting the pH to 3.5 with HCl, and then adding 95 per cent ethanol and some ethyl ether

until a precipitate was formed. This precipitate was then treated as described for the precipitate in procedure (1).

(3) By refluxing the chromoprotein, obtained after acetone treatment with N/80 NH_4OH at 100° C. for several hours. Under these conditions first the nucleoprotein was split off, followed by liberation of the nucleic acid. The latter was further hydrolyzed on continuous boiling to non-precipitable fractions.

B. *Extraction of Nucleoprotein from Purified Plastids.* Since the extracts of whole cells, obtained by the method as described above, yielded relatively large quantities of nucleoprotein (which was to be expected according to our working hypothesis), extractions were now made from purified plastids in order to eliminate any possible contamination with cytoplasmic or nuclear proteins. The methods as described under A were used beginning with the acetone extraction. After removal of the pigments, the minute disks or grana, imbedded in the stroma of the purified plastid centrifugate (see p. 767) are clearly visible. A few observations might be of interest:

(1) Alcohol extraction of the pigments was not possible. Apparently, the sucrose made the plastid wall impermeable and tough. Acetone allows extraction of the plastid pigments.

(2) In order to be sure that no pentose or phosphorus might be obtained from lecithin-like components of the chromoprotein complex, the acetone-washed preparation was not washed with chloroform, but refluxed for at least 1 hour in a chloroform-ether mixture, thereupon washed with ether, alcohol, and finally with distilled water.

(3) The nucleoprotein was extremely difficult to extract from the whole chromoprotein complex, when this complex had been beforehand precipitated either by freezing, boiling, or the addition of certain mineral salts. This indicates that any treatment that denatures the complex (for instance freezing of the material) might interfere with the tests for nucleoprotein or nucleic acid.

III. Properties of the Chromoprotein Complex

A. *Light Absorbtion in the Visible Spectrum.* In order to prove that no appreciable denaturation occurred during the extraction of the chromoprotein complex or the preparation of purified plastids, extinction curves for visible light (3900–8000 Å) were made by means of a Coleman spectrophotometer. The spectral transmittance curve of a living leaf of *Nicotiana tabacum* var. Turkish (Fig. 2 "leaf") was compared with a chromoprotein extract of the same variety (Fig. 2 "chromoprotein"), and a purified plastid suspension in water (Fig. 2 "plastid"). The living leaf was infiltrated with water under evacuation immediately before measuring and placed in water between two microscope slides (14). The chromoprotein extract was one of those kept in a refrigerator for some months, to demonstrate the stability of the extracts. The region of maximum extinction for the chloro-

phylls in all three cases was 6780–6790 Å, for the carotenoids 4400 Å. From this evidence we conclude that no considerable denaturation of the natural chromoprotein extract occurred. That *both chlorophylls and carotenoids are carried by the same chromoprotein complex* is shown by the fact that the pigments remain together throughout the whole preparative procedure, and can be separated only by denaturation of the carrier complex (*cf.* 8). For comparison a curve showing the extinction by a petroleum-ether extract of the chromoprotein has been added (Fig. 2, "petroleum ether extr.").

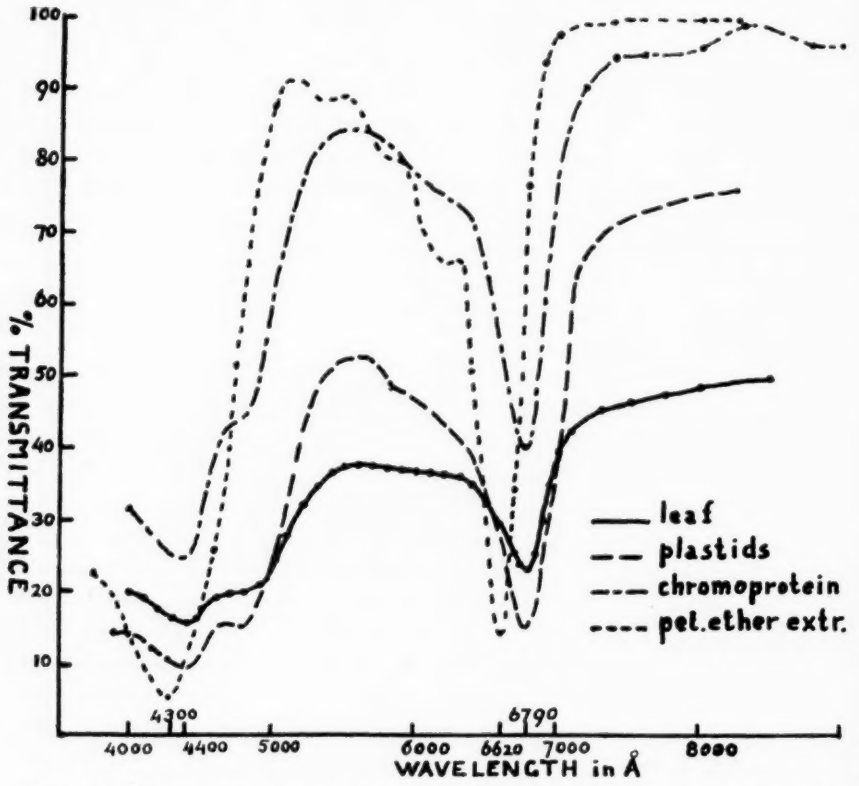

FIG. 2. Spectral transmittance curves in the visible spectrum of a living leaf of *Nicotiana tabacum* var. Turkish (unbroken curve). A purified plastid preparation of leaves of the same variety (widely broken curve), a chromoprotein solution of the same variety (dot-and-dash curve) and of a petroleum-ether solution of the same material (finely broken curve). Wavelength in Å-units, transmittance in percentage of water in the case of the living leaf and of the solvent in the others.

Since the prismatic dispersion and the light intensity of the lamp of the spectrophotometer change very gradually around 6780 Å, a very precise determination of the point of maximum extinction could be made by balancing the instrument for example at 6780 Å, and then adjusting the wavelength scale to the point where the deviation of the indicating galvanometer was minimal. In this manner the same value of maximum absorption for leaf and solutions has been determined independently by 3 workers in our

laboratory. The occurrence of carotenoids in chromoprotein extracts of spinach has already been mentioned by Smith (16) and others.

B. *Birefringence of the Chromoprotein Preparations as Compared with That of Mosaic-virus Preparations.* Chloroplasts have been reported as birefringent (10), whereas tobacco mosaic and certain other virus proteins show anisotropy of flow (2). Therefore, the purified chromoprotein extracts were tested for birefringence. Some of the purified preparations showed faint anisotropy of flow. Since the weakness of this phenomenon might be due to the occurrence of aggregates of mainly non-oriented micelles, a weak solution of sodium dodecylsulphate (0.1 per cent) or of $(NH_4)_2SO_4$ (1 per cent) was added in the proportion of 0.05 cc. per 10 cc. extract and the preparation allowed to stand for 24 hours at room temperature (about 22° C.). Strong green streaming birefringence was observed.

In order to determine whether this birefringence was due to the increased salt concentration itself or to its orienting effect the preparation was dialyzed against distilled water for 48 hours. Birefringence was still as strong as in the original sample, indicating that the salts acted only as orienting agents.

The absence of any contaminating masked virus in the extracts that, if present, might have caused birefringence was assured (1) by the method of fractionation that would have removed the more soluble virus proteins, (2) by infectivity tests on *Nicotiana tabacum* and *N. glutinosa*, which were negative, and (3) by differential precipitation of mosaic-virus protein and chromoprotein with a series of $(NH_4)_2SO_4$ concentrations.

The failure of the freshly prepared chromoprotein extracts to show streaming birefringence is apparently due to the fact that immediately after extraction the protein micelles are not oriented to form elongated compound particles. A similar situation was observed in tobacco-mosaic-virus preparations prepared from recently matured leaves shortly after infection. A lack of streaming birefringence of such virus preparations could be demonstrated by extracting the virus from young plants, infected only 12 days before extraction, following the method described previously (20), with the improvements as mentioned on page 768. These nonstreaming birefringent preparations, containing about 3.2 mg. protein per cc. of solution were colorless, only weakly opalescent, but highly infectious ("old" virus preparations are always somewhat brown). Various tests were performed with such preparations. One sample was left standing at room temperature. This developed weak birefringence in time. To a second sample sodium dodecylsulphate (to approximately 0.05 per cent) was added. Within 12 hours this developed strong streaming birefringence and also increased opalescence, which were identical in appearance to that occurring in samples of "old" virus. This sample, when kept for weeks, continued to show birefringence. A third sample was dialyzed against tapwater for 48 hours and distilled water for 48 hours. No streaming birefringence could be detected, even after 14 days standing at 4° C. A fourth nondialyzed sample

was kept at 4° C. This developed very weak streaming birefringence after some weeks standing. These observations are in accordance with Frampton's considerations on the shape and size of mosaic-virus particles (7).

Apparently, as in the case of the chromoprotein preparations, the micelles of "fresh" virus of recently infected plants *are not arranged in rod-shaped aggregates sufficiently to show streaming birefringence.*

Summarizing, we can state that removal of salt by dialysis suppresses the polar arrangement of the micelles in non-oriented preparations, whereas standing at room temperature increases the orientation slowly. Weak sodium dodecylsulphate solutions speed up the polar arrangement of the micelles.

C. *Fluorescence.* Watery solutions of the chromoprotein complex are clearly red fluorescent, although not so strongly as acetone solutions of pure chlorophyll.

In the light of the chemical and physical data presented here we can conclude that the chromoprotein complex, prepared by the methods given in this and a previous paper (20) must resemble closely the state in which it occurs in the living leaf.

IV. Properties of the Nucleoprotein Obtained from Plastids

The nucleoprotein, obtained as described under III was soluble in alkaline solutions and precipitated at pH 4.7. It was insoluble in alcohol, acetone, ether and chloroform. The protein in solution had a high buffer capacity to base on the acid side of pH 4.7. Millon's, xanthoproteic, and biuret reactions were positive. Bial's test (orcin, HCl, $FeCl_3$) yielded a blue-green color reaction, the orcin-HCl test a purple color. The ammonium molybdate-benzidine reaction for phosphorus was positive. The nucleoprotein remained in solution even after 4 days of dialysis against distilled water. The solution was brownish and opalescent. The ultra-violet absorption spectrum was determined from a sample prepared from pure plastids by digestion of the protein complex (after removal of the pigments) with 0.1 N KOH at 0° C. for about 12 hours. The digest was brought to pH 6, the non-soluble protein centrifuged out and the supernatant brought to pH 8, diluted ten times and measured. The maximum absorption band was from 2570–2600 Å (Fig. 3).[2] The curve is similar to the curve obtained by Claude (3) for the tumor fraction of mouse-brain preparations, and by Bawden for bushy-stunt virus (2).

The pure nucleic acid preparation, obtained by alkaline digestion of the nucleoprotein was precipitated by 85 per cent acetic acid. The white powder was soluble in alkali at pH 8.0. Millon's, xanthoproteic, and biuret reactions were negative, as was Schiff's fuchsin-sulphurous acid test for desoxypentose. Schiff's aniline acetate test for furfural was positive after acid hydrolysis of the nucleic acid brought about by boiling it for a few

[2] We are indebted to Mr. H. Wiseman of the Bureau of Animal Industry, U.S.D.A., for determining the absorption spectrum of this preparation.

minutes in 12 per cent HCl, indicating the presence of d-ribose. The nucleic acid is totally hydrolyzed by 0.5 N NaOH in the cold or by boiling with dilute NaOH or with 30 per cent HCl. It is insoluble in boiling ether-chloroform or in ether-chloroform-alcohol mixture, even after continued refluxing. This excluded the presence of lecithin-like substances. A nucleic

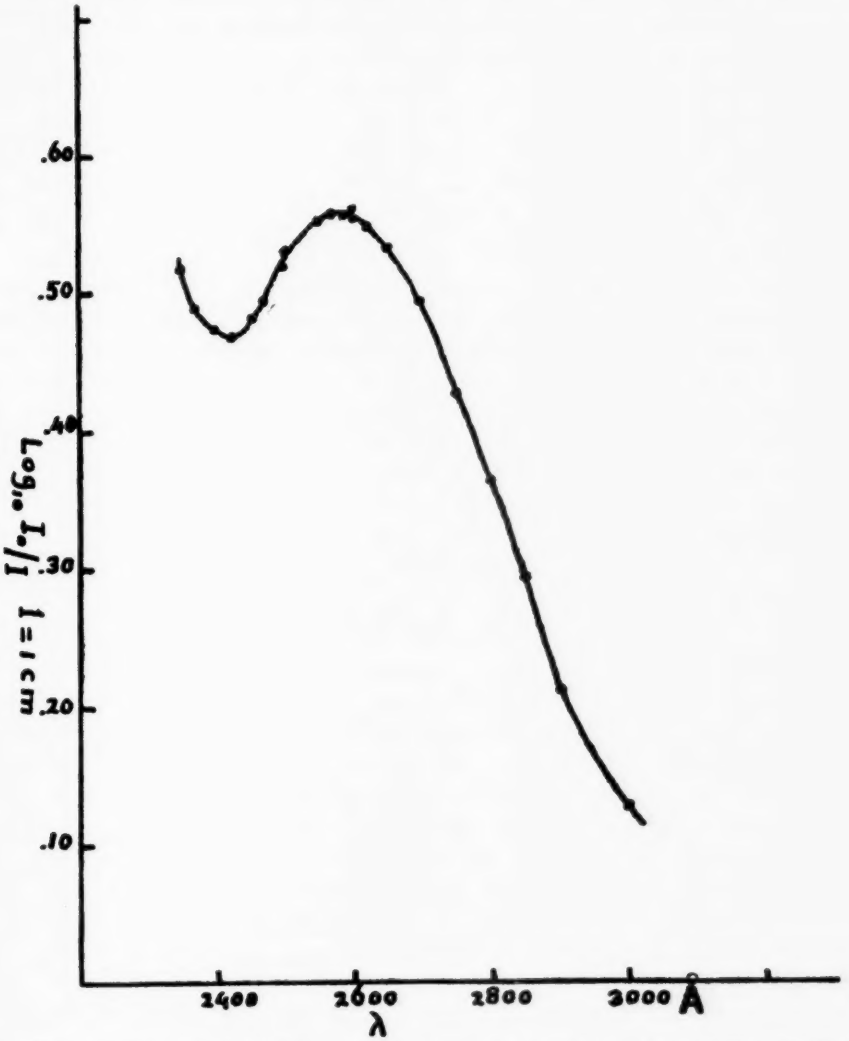

Fig. 3. The ultraviolet absorption spectrum of a nucleoprotein solution obtained from purified plastids of *Nicotiana tabacum* var. Turkish.

acid precipitate was obtained by hydrolyzing the chromoprotein complex with 0.5 N NaOH and by treating the alkaline solution of nucleoprotein (twice refluxed in ether with 5 volumes of glacial acetic acid). The precipitate was washed with 1 per cent HCl, alcohol, and ether, and then dried. After redissolving in dilute NaOH, the preparation was filtered and mea-

sured. It might have contained some nucleoprotein. The absorption curve was determined according to the usual match-point method.[3] Maximum absorption was between 2560 and 2600 Å.

DISCUSSION

A number of investigators have worked with "Lubimenko-extracts" (12), which are partly suspensions of the grana that constitute the chloroplasts, and with various chlorophyll extracts. Hubert (11) reviews the literature before 1935. He reports that, except for a ground leaf suspension, the main absorption band of chlorophyll in colloidal solutions is always found moved towards the blue. Since 1935, Stoll and Wiedemann (17), Shafer (15), Smith (16), Fishman and Moyer (6), to mention a few, have worked with modified Lubimenko extracts of chlorophyll. They mention a brown supernatant that indicates oxidation, which we have shown to cause an instability of the position of the absorption bands of the purified pigmented protein extracts. Our experiments have demonstrated that this instability is due to an insufficient prevention of the action of oxidative enzymes. Some of the samples prepared according to the methods described in this paper have been stable for more than 6 months when kept in the refrigerator at about 4° C., showing only some broadening of the absorption bands. However, as soon as some oxidation was allowed to proceed, the point of maximum absorption shifted gradually from 6780 Å and after some hours to 6750 Å.

In an earlier paper the writers (22) presented cytological and genetic evidence for the evolution of viruses from plastids or proplastids (mitochondria or chondriocontes). In this paper the chemical similarity of viruses and an essential part of the chromoprotein complex, namely a ribose nucleoprotein is demonstrated. Thus, one of the arguments in favor of the organismal origin of viruses, namely, that no nucleoprotein of the ribose type could be found in the "host" in sufficient quantity to explain the high concentration of virus protein, sometimes encountered, is invalidated.

A further parallelism between the cytological and chemical evidence for the evolution of viruses from plastid or mitochondrial ribose-nucleoproteins can be found in the gradual modification of the variegation-inducing plastid as presented in a "spectrum" of variegations (22). The first cytological evidence of plastid change occurs in mild variegations. Here light green plastids are found. Continuing along the spectrum of variegation the next change is the occurrence of light-green plastids, which turn yellow (= loss of chlorophyll), the occurrence of yellow plastids, which bleach (= loss of some carotenoid pigment), the occurrence of colorless plastids (= loss of both chlorophylls and carotenoids), the occurrence of proplastids (chondriocontes or mitochondria) only (= loss of complex plastid structure), and finally, the occurrence of small sub-mitochondrial units only. Presumably slow-moving

[3] We are indebted to Mr. H. Armstrong of the National Cancer Institute for photographing the ultra-violet absorption spectrum of this sample.

viruses, such as that causing *Abutilon* mosaic, are of such a structure, whereas viruses like that of tobacco mosaic represent only the modified monomolecular ribose nucleoprotein residues of the plastid complex.

This gradual loss of components presents a parallel with the degrees of chemical linkage that exist between the components of the chromoprotein complex as indicated, *e.g.*, by the gradual breakdown of purified plastid preparations by controlled chemical means or by aging. A treatment with acetone removes the chlorophylls first, followed after repeated washing by the removal of the carotenoids. In order to remove the lecithin-like components still more drastic treatments must be used, and, in order to liberate the free nucleoprotein, a prolonged digestion is necessary.

Following up this parallel we offer the diagram below, part of which has already been proved to be correct, as a working hypothesis that visualizes the chemical evolution of viruses from normal plastids or mitrochondria.

TABLE 1.—*Diagram indicating the chemical evolution of plant viruses from plastids or mitochondria*

Normal plastids	Plastids of Region I of the "variegation spectrum"	Mitochondria or proplastids of Region III–IV–V of the spectrum and certain complex viruses	Viruses
Chlorophylls	Carotenoids	Lipoproteins	Nucleoproteins of the ribose type
Carotenoids	Lipoproteins	Nucleoproteins of the ribose type	.....
Lipoproteins	Nucleoproteins of the ribose type	.....	.....
Nucleoproteins of the ribose type	.....	.....	.....

The animal viruses of the ribose nucleoprotein-type could similarly have derived from the chondriome, or mitochondrial-complex of the host-cell. Thus, also over the chondriome a connection might be established between certain cancer agents and viruses (*cf.* 18). In this way the variegation-inducing plastid or chondrioconte can be considered analogous to the cancer inducing mitochondrium (= chondriosome) with its limited intercellular mobility. The work of Claude (3) presents evidence for this statement, since he has demonstrated the presence of ribose nucleoproteins in the particulate components of normal and cancerous cells of chick and mouse (presumably mitochondria) and has suggested the etiologic significance of abnormal mitochondria in cancer (*cf.* also 19).

Proof for the origin of animal viruses from the mitochondria or chondriome is more difficult to furnish, than for the origin of plant viruses, since the mitochondria in animals remain small and are never "tagged" like the modified plastids of plant-variegations. A further study of the causes leading to the gradual change of the nucleoprotein complex in the modified plant

plastids might lead to a better understanding of various animal viroses and cancers, as well.

SUMMARY

An extraction method for the chromoprotein complex from plastids is presented.

Some conditions for the occurrence of streaming birefringence of the complex are given.

For comparison some conditions for the occurrence or absence of streaming birefringence of tobacco mosaic virus protein are presented.

The resemblance of the chromoprotein complex to the complex as it occurs in the living state is demonstrated by analysis of the spectral absorption of extracts and living leaves and by some of its chemical and physical characteristics.

Various reactions characteristic of ribose nucleoprotein are given by a protein fraction of the chromoprotein complex of purified plastids of *Nicotiana tabacum*.

Some evidence for the evolution of plant virus nucleoproteins from the nucleoproteins of the plastids is presented, which suggests the possibility of a similar evolution of certain animal viruses from mitochondria, and a parallelism of variegation-inducing plastids and cancer-inducing mitochondria.

The authors wish to express their indebtedness to Dr. N. L. Drake, Head of the Department of Chemistry, University of Maryland, for his evaluation of the various chemical procedures used, and Miss H. Ryan for her help in preparing the manuscripts of the two papers.

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PRODUCTIVITY OF MOSAIC-RESISTANT REFUGEE BEANS

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INTRODUCTION

Since the variety Stringless Green Refugee has become a widely used snap bean (*Phaseolus vulgaris* L.) for canning in northern United States, its extreme susceptibility to common mosaic (*bean virus 1* Pierce) has often been a limiting factor in production. The successful control of the disease in this type of bean began with the discovery of an immune plant in the variety by Ralph Corbett of the Sioux City Seed Company at Billings, Montana, in 1929. The progeny of this plant was also completely immune, and the subsequent variety known as Corbett Refugee has served as a parent for breeding purposes. In itself Corbett Refugee, although similar to Stringless Green Refugee in many respects, was unsuitable in others to replace the latter. The first 2 varieties developed from Stringless Green Refugee and Corbett Refugee were derived from a cross made in 1930 by Pierce and Walker (8). These were designated as Wisconsin Refugee and Idaho Refugee and were introduced to the trade in 1934. A third variety was introduced as U. S. No. 5 Refugee by Wade and Zaumeyer (11) in 1935. In 1941 Anderson (1) announced two new varieties, Sensation Refugee 1066 and Sensation Refugee 1071, derived from a cross of Idaho Refugee with Stringless Green Refugee and with U. S. No. 1, respectively.

By 1937 the first three of the resistant varieties mentioned had reached commercial volume. Preliminary experimental lots of the last two varieties were made available by the introducers. The purpose of the investigation reported herein was to study the productivity, pod type, and canning quality of these new varieties in comparison with mosaic-susceptible Stringless Green Refugee. Some attention was also given to the effect on yield of an inherited variegation in some of the varieties.

METHODS AND MATERIALS

Trials were conducted at Madison, Wisconsin, in 1937, 1938, 1939, and 1942, and at Racine and Green Bay, Wisconsin, in 1939. Single-row plots, 25 feet long, were planted in randomized and replicated plots at Madison and Racine. Double-row, non-replicated plots, 150 feet long, were planted at Green Bay. Pods were picked at regular intervals and weighed. Since no significant differences occurred between various commercial lots of a given variety, only the averages for each variety were presented here. Commercial lots of Stringless Green Refugee usually contain a majority of seeds that carry *bean virus 1*. Before mid-season most plants in this variety became diseased. In order to study the effects of the disease on this variety, a virus-free stock was built up by growing a commercial stock in the greenhouse where infected plants could be eliminated and dissemination

of the virus prevented. The stock thus secured was increased out of doors in an isolated area and again rogued thoroughly. The mosaic-free stock was used in the yield trials of 1939 and 1942.

At certain pickings in 1939 random samples from each lot were graded according to standard sizes based on the maximum diameter of the pod. Since the ideal Refugee bean pod for canning is circular in cross section, measurements also were made of the diameter of the cross section taken from cheek to cheek and from suture to suture. The S/C factor was then obtained by dividing the average cheek diameter into the average suture diameter. Thus, as pods tended to become flat this factor exceeded 1.00. The writers are indebted to Dr. J. H. Torrie of the Department of Agronomy, University of Wisconsin, for assistance in selection of plot designs and in setting up data for analysis.

EXPERIMENTAL RESULTS

Occurrence of Mosaic-like Variegation

Corbett Refugee contains a chlorophyll-deficient character that has been retained in Wisconsin Refugee and Idaho Refugee. It is manifested in various forms some of which are readily confused with mosaic. Mottling occurs in one or more leaflets of the trifoliate, sometimes in the unifoliate leaf, and very commonly it is confined to one side of the midrib, causing dwarfing, which may be so extreme as to result in pronounced unilateral distortion. Occasional plants may show extreme deformity of most leaves while pods are distorted, misshapen, and disfigured by necrotic areas. On the other hand, in a large share of the plants affected the abnormality is confined to 1 or 2 leaves which may drop early, leaving the plant normal for the remainder of the season.

Harrison and Burkholder (3) and Horsfall, Reinking and Burkholder (4) described this abnormality in New York and suggested it to be virous in nature, since it appeared first in the younger leaves. Zaumeyer (12) has shown it to be a hereditary characteristic, and this has been confirmed repeatedly by the writers. Other hereditary chlorophyll deficiencies in bean have been described (5, 6, 7, 9) which might be confused with mosaic. None of these seem to conform with the character found in strains of Refugee in which Corbett Refugee has been a parent. Zaumeyer (12) has presented evidence to show that it consists of 2 very similar types of variegation, and that one type of variegation is controlled by 2 complementary factors. However, no progenies breeding true for this character were secured indicating either that the double recessive is lethal or that modifying genes prevent or obscure the expression of the character in some plants of the double recessive progenies. Wade (10) has recently analyzed a second type in U. S. No. 5 Refugee very similar to that found in Corbett Refugee, Wisconsin Refugee, and Idaho Refugee. He suggests that this may have arisen in U. S. No. 5 Refugee as a result of a chance cross with Corbett Refugee. He interprets that this type of variegation may be due to any of

3 recessive genes, while the normal is attributable to the complementary action of 3 dominant genes.

Certain studies were made of single-plant progenies from plants of Wisconsin Refugee and Idaho Refugee with no abnormality and from plants showing various grades of variegation. No attempt has been made to distinguish between the 2 types indicated by Zaumeyer (12) and Wade (10). Selections made in 1939 were tested at Madison in 1940. The results in table 1 show that an average of 20 per cent of all plants were affected in the commercial Wisconsin Refugee stock, and approximately the same percentage occurred in progenies from plants selected as free from variegation. However, progenies from moderately and severely affected parents showed corresponding increases in percentages of total plants affected and in relative proportions of moderately and severely affected individuals. Wade (10) points out that in field planting of Corbett Refugee at Charleston, South Carolina, only about 25 per cent showed variegation,—in such cases the degree was slight. Under greenhouse conditions, however, all Corbett Refugee plants were variegated, and the mean was very close to that of the three lines carrying double recessives. Further evidence that variegation in Wisconsin Refugee is influenced by environment is shown in a greenhouse experiment conducted during winter months at Madison, Wisconsin.

TABLE 1.—*The occurrence of inherited variegation in progenies from plants selected from Idaho Refugee and Wisconsin Refugee and in commercial Wisconsin Refugee*

Degree of abnormality in parent plants	Number plants in progenies tested	Percentage of plants showing degree of abnormality indicated			
		None	Slight	Moderate	Severe
None	118	83	10	3	4
Moderate	173	29	18	22	31
Severe	165	15	7	20	58
Wis. Refugee (Comm.)	149	80	9	9	2

Plants from commercial Wisconsin Refugee and from seed selected from chlorophyll-deficient plants of that variety were grown in the greenhouse at 4 temperature levels: 16, 20, 24, and 28° C. The results, given in table 2, show that the lot grown from seed of affected parents contained a high percentage of affected individuals at 16, 20, and 24° C., while the abnormality was very decidedly suppressed in this group and in commercial Wisconsin Refugee at 28°. It may be possible, therefore, for variation in the amount of this chlorophyll aberration to be found in fields from the same lot of seed due to temperature variations resulting from differences in planting date, season, and location. Furthermore, it was found necessary to make frequent counts on this character to record all affected plants since only 1 or 2 leaves might show the abnormality, while earlier and later leaves remained normal. It is important, therefore, that these facts be taken into account in the genetic analysis of this character. By selection within Idaho Refugee, lines free of

this character have been obtained. No evidence of this abnormality was noted in lots of Stringless Green Refugee, U. S. No. 5 Refugee, Sensation Refugee 1066, and Sensation Refugee 1071 used in these experiments.

TABLE 2.—*The occurrence of variegation in commercial Wisconsin Refugee and in progenies from affected plants in this variety when grown in the greenhouse at different air temperatures at Madison, Wisconsin, in December, January, and February*

Average air temperature of greenhouse	Progeny from affected plants of Wisconsin Refugee		Commercial Wisconsin Refugee	
	Plants tested	Plants affected	Plants tested	Plants affected
°C.	*Number*	*Per cent*	*Number*	*Per cent*
16	40	60.0	33	15.1
20	51	64.7	35	17.1
24	52	50.9	30	16.7
28	37	8.1	32	3.1

Total Yields

The average yields for each strain or variety are given in table 3. In 1937 commercial Stringless Green Refugee gave the lowest yields, while Wisconsin Refugee was consistently intermediate between it and Idaho Refugee. U. S. No. 5 Refugee yielded closely to the latter in both seasons, while in 1938 Sensation Refugee 1066 and Sensation Refugee 1071 produced much more heavily than all others. In 1939 essentially the same order was maintained at 3 locations in the State with the exception that Wisconsin Refugee fell below Stringless Green Refugee at Madison. Idaho Refugee, U. S. No. 5 Refugee, Sensation Refugee 1066 and 1071 were each significantly higher in production than Wisconsin Refugee at this location, but there was no significant difference between the 4 highest yielders. At Racine these 4 were again highest, but only one (Sensation Refugee 1071) was significantly higher than Wisconsin Refugee. At Green Bay, where only a single plot per variety was planted, the Wisconsin Refugee yield was higher than that of either Idaho Refugee or U. S. No. 5 Refugee, while Sensation Refugee 1066 and 1071 were again the highest yielding lots.

It is of special interest to note the comparative yields of Stringless Green Refugee from commercial and from specially selected mosaic-free seed. These were compared in 1939 and in 1942. In the former year mosaic occurred in a large percentage of the plants from the commercial seed, while those plants from mosaic-free seed remained free from visible current-season infection until well into the harvest. In 1942, by contrast, there was early dissemination of the virus from the commercial stock to the selected stock with the result that both were severely affected before harvest. In 1939 the mosaic-free stock produced as heavily as Idaho Refugee, U. S. No. 5 Refugee, Sensation Refugee 1066, and Sensation Refugee 1071, and significantly higher than the commercial stock. In 1942, when the virus was disseminated early and the disease was equally severe in both lots, the selected lot dropped in yield close to the level of the commercial stock.

TABLE 3.—*Yield of mosaic-resistant and mosaic-susceptible varieties of bean of the Refugee type in 1937, 1938, 1939, and 1942*

Variety	Yield in pounds per acre at					
	Madison in				Racine in	Green Bay in
	1937	1938	1939	1942	1939	1939
Stringless Green Refugee (Commercial)	2160	4300	7457	6570	6458	4770
Stringless Green Refugee (mosaic-free seed)	.....	.....	10085	6711	8256	5880
Corbett Refugee	.....	7380	.....	.....	.....	.....
Wisconsin Refugee (Commerial)	3058	5400	7066	.....	7727	6180
Wisconsin Refugee (selected for variegation)	.....	5920	8290	.....	7426	.....
Idaho Refugee (Commercial)	4883	7120	9736	11224	8542	5675
Idaho Refugee (free from variegation)	.....	.....	.....	11813	.....	.....
U. S. No. 5 Refugee	4931	6570	10174	10743	7939	5945
Sensation Refugee 1066	.....	9220	11282	9843	8265	7410
Sensation Refugee 1071	.....	9080	10052	12807	12808	6900
Difference required for significance (19:1)	855	1280	1520	3050	1220	.....

In 1938 and 1939 a lot of Wisconsin Refugee was included that had been derived from seed selected from plants showing the variegation character described above. About 85 per cent of the plants in this stock showed the character. It is to be noted that no significant difference occurred between the yield of this lot and that of the commercial stock of the variety. In 1942 a stock of Idaho Refugee, free from this variegation as a result of several years' selection, was included. The yield of the variegation-free stock was not significantly different from that of the commercial stock of the variety. It may be concluded from these data that the variegation character has little or no influence upon yield. Its chief detrimental effect is the fact that occasional misshapen pods unsuitable for canning occur. The varieties possessing this character can and should be rid of it by selection.

Rate of Production

The rate of production as shown by the yield at various picking dates is of great importance in evaluating a variety for canning purposes. In figures 1 and 2 are given the yield curves for each variety tested at Madison in 1937, 1938, 1939, and 1942. The season of 1937 was very dry in late July and throughout August. In 1938 and 1942 the moisture supply was ample and well distributed during the picking season. In 1939 the rainfall, though not heavy, was well distributed, and in this year it was supplemented at Madison by overhead irrigation. This resulted in a longer picking period, so that harvest continued until September 11, although only yield data through August 28 are given in figure 1.

The commercial stock of Stringless Green Refugee rose gradually to a peak after mid-season in each year and with Wisconsin Refugee was usually

latest in initial yields of substantial size. In Corbett Refugee, tested only in 1938, the first pick also coincided with Stringless Green Refugee and the yield rose gradually to a peak about mid-season. Idaho Refugee and U. S.

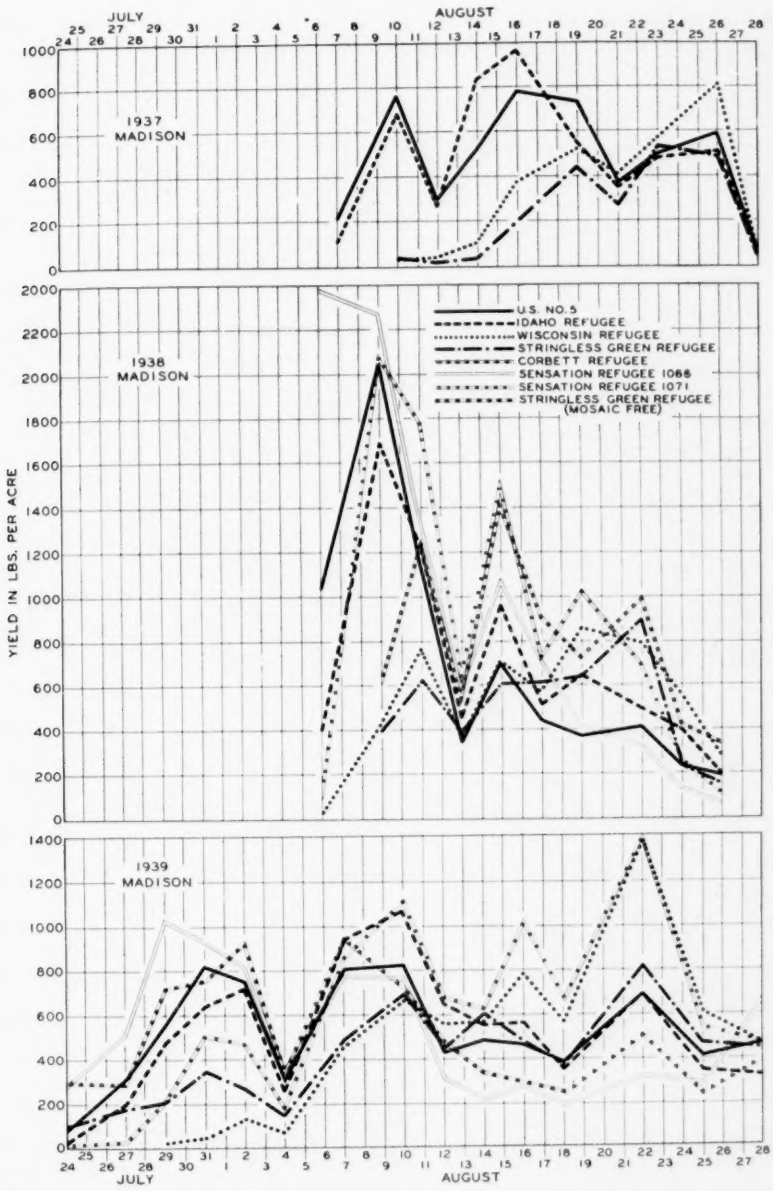

FIG. 1. Rate of production of resistant and susceptible varieties at Madison, Wisconsin, in 1937, 1938, and 1939.

No. 5 Refugee, on the other hand, rose to early harvest peaks. Thus, it might appear that the former of these was much earlier in initial pick and harvest peak than either parent, Stringless Green Refugee or Corbett

Refugee. However, mosaic-free Stringless Green Refugee when grown quite free from mosaic in 1939 produced a yield curve very similar to that of Idaho Refugee, and one that is undoubtedly the heritable yield characteristic of the variety.

Wisconsin Refugee, although it usually yielded on a curve nearly parallel with Stringless Green Refugee, being free from the influence of mosaic, is actually a later variety. Thus, of the two varieties derived from the cross between Stringless Green Refugee and Corbett Refugee, one, Wisconsin Refugee, resembles in maturity and yielding habit the resistant parent, while the other, Idaho Refugee, resembles the susceptible parent.

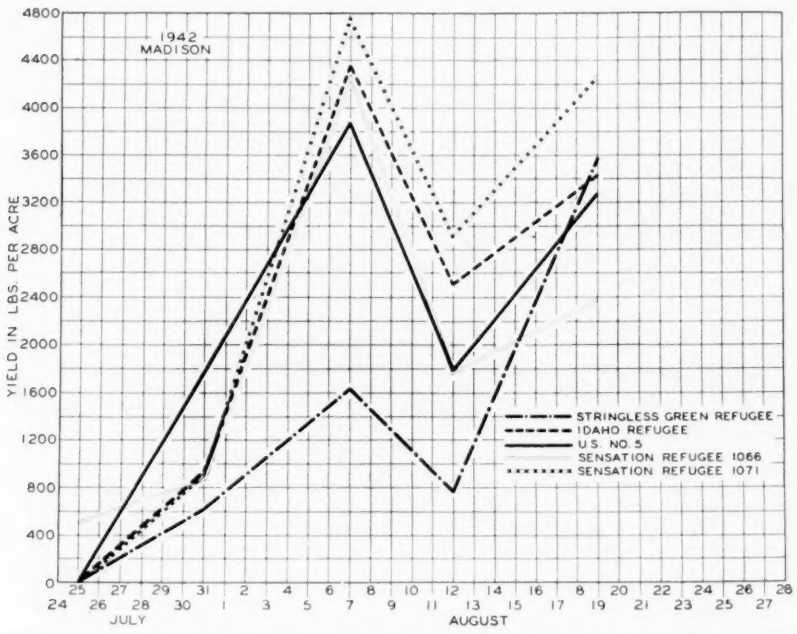

FIG. 2. Rate of production of resistant and susceptible varieties at Madison, Wisconsin, in 1942.

Sensation Refugee 1071 reached an early peak along with U. S. No. 5 Refugee, Idaho Refugee, and mosaic-free Stringless Green Refugee in 1938 and 1942, but the production curve rose more gradually in 1939. Sensation Refugee was usually ready for the first pick 4 to 7 days ahead of the other strains tested.

The mosaic disease causes some blossom-drop, stunts the plant, and delays maturity of pods. The effect of this disease on the rate of production of Stringless Green Refugee is shown when the picking curves for 1939 and 1942 are compared (Fig. 3). It has been pointed out that in the former year the mosaic-free stock remained free from disease until late in the season, while in 1942 plants from the identical lot became infected early through current-season spread. In 1939 the planting from mosaic-free seed reached

an early production peak while in 1942 when the plants were affected early it reached a late-season production peak along with that usual for the commercial stock. It appears that the high productivity of certain of the resistant varieties is due primarily to the control of mosaic through introduction of the resistant character by hybridization rather than by the introduction of any other factors that induced higher yields.

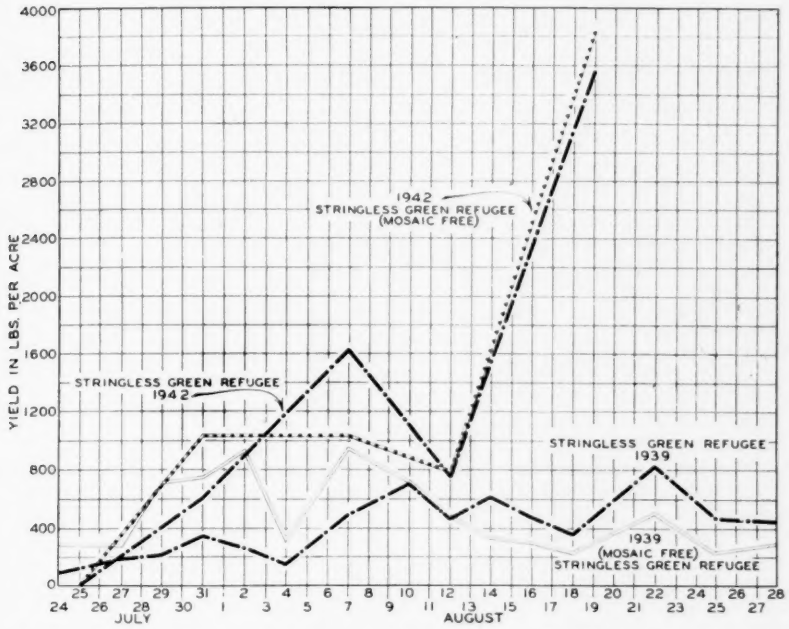

FIG. 3. Comparison of commercial Stringless Green Refugee production rate with that of the same variety grown from mosaic-free seed. In 1939 the latter remained free from symptoms for most of the season and reached a production peak much earlier than the former. In 1942, when mosaic spread early throughout the plantings, both stocks reached a production peak late in the season.

Relative Pod Size

At several pickings at Madison in 1939 random samples from the harvested pods were taken from each variety and sorted into the standard-size classes used by canners. The results in table 4 show that the crop was picked so as to give a large proportion of small sizes, as desired for the whole bean pack, for which this type is particularly suited. It is to be noted that there was little variation between varieties in the 3 and 5 sizes. Most lots were quite similar to Stringless Green Refugee in 1–2 and 4 sizes. U. S. No. 5 Refugee was significantly lower than the former and Idaho Refugee in the percentage of pods in the 1–2 size.

Relative Pod Shape

It is highly desirable that beans of this type be practically circular in cross section. In 1939 random samples of 25 pods were taken from 2 pick-

ings of each lot at Racine and Madison, and the S/C ratio was determined. The data secured are given in table 5. These results show that the pods were significantly flatter at each station in the first as compared with the second picking. However, when Stringless Green Refugee is compared with any other variety at any one of the pickings, the difference is not significant, except in one picking of U. S. No. 5 Refugee. It may be concluded, therefore, that all of the mosaic-resistant varieties tested are equal in this important character to the mosaic-susceptible parent variety.

TABLE 4.—*Relative proportion of standard pod sizes at 5 pickings at Madison in 1939*[a]

Variety	Averages of percentage of pods by weight in the indicated size class			
	1–2	3	4	5
Stringless Green Refugee (Commercial)	46	36	15	3
Stringless Green Refugee (mosaic-free seed)	39	36	20	5
Idaho Refugee	46	31	21	2
U. S. No. 5 Refugee	33	38	25	4
Sensation Refugee 1066	38	37	22	3
Sensation Refugee 1071	40	34	23	3
Wisconsin Refugee[a] (Commercial)	48	34	17	1
Wisconsin Refugee (free from variegation)	43	34	22	1
Difference required for significance (19: 1)	11	[b]	[b]	[b]

[a] Wisconsin Refugee was later in maturity than the other varieties; therefore, only 3 pickings of lots of this variety were included, and it was not included in the analysis of variance.

[b] Differences not significant.

TABLE 5.—*Pod shape as indicated by the S/C factor*

Variety	S/C factor at station and dates indicated					
	Racine			Madison		
	Aug. 17	Aug. 20	Means	Aug. 18	Aug. 25	Means
Stringless Green Refugee (Commercial)	1.08[a]	1.01	1.05	1.07	1.01	1.04
Stringless Green Refugee (mosaic-free seed)	1.07	1.00	1.04	1.06	1.10	1.08
Idaho Refugee	1.04	1.00	1.03	1.02	0.99	1.00
U. S. No. 5 Refugee	1.14	1.03	1.09	1.06	0.94	1.00
Sensation Refugee 1066	1.07	1.02	1.05	1.05	1.05	1.05
Sensation Refugee 1071	1.04	1.00	1.02	1.01	0.97	0.99
Wisconsin Refugee (Commercial)	1.05	1.03	1.04	1.03	0.99	1.01
Wisconsin Refugee (selected for variegation)	1.05	1.04	1.05	1.05	0.97	1.01
Means	1.07[b]	1.02	.....	1.04	1.00	.....

[a] Difference required for significance (19: 1) between varieties at a station on a given date 0.07.

[b] Difference required for significance (19: 1) between dates at a given station 0.03.

Canning Quality

In 1939 samples of ungraded whole pods from one of the mid-season pickings of each variety at Green Bay were processed at a commercial

cannery. Samples drawn from the pack were graded by a representative of the Bureau of Agricultural Economics, U. S. Department of Agriculture. All showed a maximum score as to clearness of liquor. All graded within 80 per cent of the maximum for uniformity of color. Each was graded at 95 per cent of the maximum score for flavor. It is thus evident that all the mosaic-resistant varieties tested were equal in canning quality to the mosaic-susceptible Stringless Green Refugee.

Discussion and Summary

The control of disease through the development of resistant varieties commonly involves the outcrossing of a susceptible variety well-adapted to a certain environment and a commercial need, with a less desirable highly resistant strain. Such was the case in the improvement of Stringless Green Refugee bean for resistance to common mosaic. The present investigation was planned to compare the susceptible variety with 5 resistant varieties as to yield, pod shape, rate of production, and canning quality.

By comparison of the commercial stock of the susceptible variety with one in which the seed was virus-free, it was shown that the disease not only reduces the yield but distinctly alters the production rate. Four of the resistant varieties, Idaho Refugee, U. S. No. 5 Refugee, Sensation Refugee 1066, and Sensation Refugee 1071, were closely similar to the mosaic-free susceptible variety in quantity of yield and rate of production. Sensation Refugee 1066 was consistently earlier than all others in production of pods at the canning stage. Wisconsin Refugee was distinctly later in maturity than the other 4 resistant varieties and usually lower in total yield. Its production increased gradually to a peak late in the harvest period; and in this respect it was similar to the behavior of the susceptible variety when the latter was affected early by mosaic.

No significant differences of importance were found between the resistant varieties and the susceptible one as to pod shape and size. All had equally high canning quality.

All of the desirable characteristics of the original susceptible variety seem to have been retained in the resistant forms. Among the last-mentioned is a range of maturity from that of the earliest, Sensation Refugee 1066 to the latest, Wisconsin Refugee.

A variegation inherited from the resistant parent occurs in Wisconsin Refugee and Idaho Refugee. Its expression is repressed by high air temperatures. Although it did not have a significant influence upon yield, it should be removed from these varieties by selection, since affected plants produce some distorted pods undesirable for processing.

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THE RUSTS OF SAFFLOWER[1]

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.) has been cultivated from ancient times for the dye extracted from the flowers and the oil present in the seed (7). It is still cultivated extensively in India and Egypt for its oil seed and to a small extent in China, Japan, Turkestan and parts of Europe (16). Since the revolution, safflower evidently has been tested in widely separated regions of the Soviet Union as a source of oil. The plant also has been under trial since 1925 in the United States (16) and still more recently in Canada.

Upon the discovery of *Puccinia carthami* on safflower in Canada, a search was made to determine the history of the rust and its geographical distribution. The scattered nature of the literature appears to warrant the publication of information available on this and the other rusts of safflower.

PUCCINIA CARTHAMI

Puccinia carthami Corda was observed for the first time in Canada in 1942. It was collected on safflower in the variety plots at Saskatoon, Saskatchewan, on September 3, by R. C. Russell (S *1264*) (Dept. Agr. Ottawa Myc. Herb. *12016*). Rust infection was light to moderate on several lines or varieties. Subsequently, it was learned that a similar observation was made by T. C. Vanterpool, who examined the same plots on August 15. The rust, however, was collected as early as July 28 at Morden, Manitoba, by W. E. Sackston (Dept. Agr. Ottawa Myc. Herb. *12017*). The severity of infection on the same plants was estimated by B. Peturson to be 20 per cent on August 26. Uredinia and telia, the only known spore stages for the species, develop in small scattered pulverulent pustules (23, p. 33) and are present in both specimens. The material was found to agree well with the description and figure published by Arthur (1, p. 349). The identification was confirmed by G. B. Cummins, Purdue University, Lafayette, Indiana, who compared part of the Saskatoon collection with the single American collection from Massachusetts, and with three collections from the Old World in the Arthur Herbarium. Portions of the latter were afterwards made available for study. Certain specimens were also kindly

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[3] The author wishes to acknowledge his indebtedness to those who aided him in this study, particularly to Dr. G. B. Cummins for advice and help with the literature, to Dr. M. I. Timonin for translating parts of Tranzschel's work from the Russian, and to Dr. R. C. Russell for supplying the data obtained by him from an examination of samples of safflower seed.

loaned by D. H. Linder from the Farlow Herbarium of Cryptogamic Botany, Harvard University, Cambridge, Mass.

From an examination of seed samples, details of which are reported later, the rust evidently occurred at Brooks, Alberta, in 1939; at Ottawa, at least in 1940; and at Indian Head, Sask., in 1941 and 1942. However, the rust was not observed at Winnipeg, Man., where the safflower plots at the University were examined in September, nor was it found on seed of the 1942 crop from Lethbridge, Alta., or in the plots during a general survey for disease carried out at the Station in the same year.[4]

The occurrence of this rust in Canada is of considerable interest, as it has been reported only once previously in North America, and because safflower may yet become a crop of some importance in Canada and the United States. The sole American record is based on a collection made on *Carthamus tinctorius* at Cottage City, Mass. (2, p. 512), in 1895 (1, p. 349). Although other diseases have been found on the crop during the recent trials in the United States (16), no mention of the rust is made. No additional specimens or records are known to the Division of Mycology and Disease Survey, Bureau of Plant Industry Station, Beltsville, Maryland.[5]

Puccinia carthami was described by Corda in 1840 on *Carthamus tinctorius* collected at Prague in Bohemia (2, p. 512). The Sydows (22, **1**: 35) record, in 1902, its collection at Ober Glogau in Silesia, at Saaz and Prague in Bohemia, near Giza in Egypt by G. Schweinfurth, and in eastern India and Japan. Two specimens on this host from Japan have been examined: Botanical Gardens, Tokio, Y. Tanaka, June, 1888 (Farlow Herbarium); Sapporo, Prov. Ishikari, Naohide Hiratsuka, July 21, 1926 (Dept. Agr. Ottawa, Myc. Herb. *12018*, ex Herb. Arthur *F8614*). As noted by Tranzschel (23, p. 402), it would appear that the rust occurs widely wherever the safflower is cultivated. He records it on safflower from the following localities in the Soviet Union—Azov-Black Sea region: Rostov, Maikop, Kayal, Armavir, Otrada Kubanskya; Armenia; Azerbaijan: Kirovabad; Uzbekistan: Tashkent (23, p. 372). The rust is known in Transylvania, where it was collected near Cluj, distr. Cojocna, by M. Péterfi, Sept. 4, 1918 (Fl. Roman. *204*, copy in Farlow Herb. examined). It also has been recorded on *Carthamus tinctorius* in India at Pusa and in the Punjab (3, p. 65), in Cyprus (14, p. 15), and again in Egypt (19, p. 130; 11, p. 37). Its occurrence in Italy is based on a single brief mention by Bagnis (24, p. 96), and, although the host is cultivated in different regions of the Iberian peninsula, and is subspontaneous in others (6, pp. 281, 377), it is uncertain whether or not the rust has been collected in Spain.

Puccinia carthami has been reported also on: *Carthamus ?creticus* (probably = *C. lanatus* L.) by Bagnis (24, p. 96); *dianius* Sch. (= *Carduncellus caeruleus* (L.) Less.) in Spain (6, p. 378); *C. glaucus* Bieb. at Kyrenia, Cyprus, R. M. Nattrass *810*, May, 1936 (14, p. 15), on the label

[4] Letter from Dr. M. W. Cormack, Edmonton, Alta., Dec. 22, 1942.
[5] Letter from Mr. J. A. Stevenson, Nov. 27, 1942.

of the specimen examined (Mycol. Herb., Ottawa, *12020;* ex Herb. Arthur *F9436*) the host is given, however, as *C. lanatus* L.; *C. lanatus* L. at Canoblas N. S. W. (20, p. 22); *C. oxyacantha* Bieb. at Kangra, Punjab, India, E. J. Butler, May 4, 1905 (21, p. 256), the copy of P. Sydow, Ured. *2116* in the Arthur Herbarium examined (Mycol. Herb., Ottawa, *12019;* ex Herb. Arthur *F8615*); in the Soviet Union in Azerbaijan: Jebrail, Kirovabad, Gheokchay; Turkmeniya: Kopet Dagh (23, p. 372).

A doubtfully distinct rust, *Puccinia kentrophylli* Syd., is reported on *Carthamus lanatus* L. (*Kentrophyllum lanatum* (L.) DC.) in the Soviet Union in Turkmeniya: Ashhabad, Kopet Dagh; Tadjikistan: Gorny Zeravshan (23, p. 372); *Kentrophyllum creticum* (probably = *Carthamus lanatus* L.), and *K. syriacum Boiss.* (= *C. glaucus* Bieb.) in Crete (22, **1**: 104).

The host genus *Kentrophyllum* is no longer considered distinct from *Carthamus.* Accordingly, if *Puccinia kentrophylli* is to be maintained as a species distinct from *P. carthami,* sound morphological differences are required for their separation. To throw some light on this point, the descriptions of the 2 species as they are given by the Sydows (22, **1**: 104 and 35) and Arthur and Mains (2, p. 512) were compared with the rust in the 7 collections studied. As a result of the comparison, there appeared to be no sound basis for their separation. Sections of the sori disclosed uredia, with teliospores entirely absent, and telia, containing at least some urediospores. In one small unbroken sorus, several urediospores were present, but teliospores were already beginning to form. It is suggested that the possible absence of uredia in the original material, from which *P. kentrophylli* was described, was due to the collection being scanty or because, with the advance of the season, only telia were being formed on the green parts of the plant as a result of secondary infection. The telia of *P. kentrophylli* are given as pulverulent, but remaining covered by the epidermis. Generally, the pustules are soon naked, but the ruptured epidermis is usually evident, and some sori are still covered. The pedicel of the teliospore in *P. kentrophylli* is said to be hyaline, slender, up to 50 μ in length, but deciduous, while the teliospore pedicel in *P. carthami* is reported to be hyaline and very short by the Sydows in contrast to colorless, delicate, fragile, mostly deciduous by Arthur and Mains. In sections of the sori, the pedicel was found to be variable in length and may reach 65 μ or more. It is very delicate and soon collapses, except for a short portion, 3–7 μ in length, adjacent to the spore. This piece, usually with only a small part of the collapsed pedicel adhering, is detached with the spore. The basal portions persist, however, after the spores have disappeared and are not to be confused with paraphyses, for, in young sori, no free hyaline elements are present. Spores in all stages of development, with the younger nearer the floor of the sorus, may be seen crowding through them. The urediospore pedicels similarly persist after the spores have become detached. Considerable variability was observed in the size of the teliospores, the pigmentation and markings of the spore wall, etc., from collection to collection,

but these characters did not seem to be correlated in any way. It may be noted that the wall of the urediospore is often thicker at the point of attachment to the pedicel than elsewhere. In all the collections, 3, or occasionally 4, equatorial pores are present. Contrary to later observers, but 2 pores are reported by the Sydows for *P. carthami.* The correctness of their statement can only be settled by a re-examination of the older European collections available to them.

A closely related species is *Puccinia carduncelli* Syd. reported on *Carduncellus caeruleus* (L.) Less. in Sicily (22, **1**: 33). This rust was studied from a specimen on the above host from Orleansville, Algeria, L. Trabut, June 20, 1912 (R. Maire, Myc. Bor.-Africa. *86,* copy in Farlow Herb.). Only telia were found. The teliospores resemble closely those of *P. carthami,* but are remarkable for their great variability in size and shape. A few urediospores were also located. They were ovate, rather than ellipsoid, with two superequatorial germ pores placed opposite each other. The rust is apparently the same as one described by Malençon[6] from specimens collected on *Carthamus calvus* (Boiss. and Reut.) Bott. at Dayet Ahoua (Moyen-Atlas) June 19, and Oujda on June 24, 1936, in Morocco, but referred by him to *P. carthami.* Maire and Werner[7] transferred the rust to *P. carduncelli.* There is a suggestion that Boissier and Reuter were correct when they originally described the host as *Carduncellus calvus.* It is very probable that the rust reported on *Carthamus dianius* (= *Carduncellus caeruleus*) from Spain (6, p. 377) belongs here rather than in *P. carthami.*

OCCURRENCE OF TELIOSPORES OF PUCCINIA CARTHAMI ON SAFFLOWER SEED

As safflower is a cultivated, rarely adventive plant in several countries of the world, including Canada and the United States, the scattered appearance of *Puccinia carthami* in these countries must be due to its chance introduction as suggested by the Sydows (22, **1**: 36). In Europe, there is the possibility that a rust on some native host might have spread to safflower, but such a possibility seems very remote in North America. It would appear more probable that spores carried on the seed might cause infection of the plant. To determine whether spores were present or not on safflower seed, 14 samples of seed were obtained from W. G. McGregor, Cereal Division, Central Experimental Farm, Ottawa. A test tube was filled with about 1½ inches of seed, water was added to fill about 3 inches of the tube. The tube was then shaken vigorously. The washings, about 10 cc., were pipetted off and centrifuged. The supernatant liquid was decanted off. The sediment was shaken in 2–3 drops of water and poured out on a slide. The latter was gently heated to drive off most of the water, 2 drops of lactophenol were added and the slide heated further to clear the spores.

[6] Malençon, G. Notulae mycologicae maroccanae, II. Rev. Mycol. 1–2: 263. 1936.
[7] Maire, R., and R. G. Werner. Fungi maroccani. Catalogue raisonné des champignons connus jusqu'ici au Maroc. Mem. Soc. Sci. Maroc. 45, p. 62. 1937 (1938).

TABLE 1.—*Occurrence of teliospores of Puccinia carthami on safflower seed examined at Ottawa*

Designation	Place of origin	Place sample grown	Year	Number of spores
Type 1	India	Lambeth, Mont.	.....	0
Type 7	"	" "	.....	0
Type 14	Russia	" "	.....	3
Type 25	Pusa, India	" "	.....	0
C.D. 2651	United Provinces, India	India	.....	0
C.D. 3230	Hungary	Brooks, Alta.[a]	.....	2,500[b]
C.D. 3230	"	Ottawa	1940	27, 39[c]
C.D. 3230	"	"	1941	0, 0[c]
C.D. 3230	"	"	1942	0, 8, 1[c]
Type 1		"	1942	0
Type 6	Saratov, Russia	"	1942	0
Type 14		"	1942	0
Type 25		"	1942	0
C.D. 2650	United Provinces, India	"	1942	0

[a] Seed from D. Demetrovits, Brooks, Alta., April 15, 1940.
[b] Estimated number. An average of 21 spores was counted in each of 6 low-power fields. Urediospores were also abundant.
[c] Duplicate or triplicate examinations.

As Table 1 shows, the seed received from Brooks, Alta., was carrying a heavy spore load. It is believed that the original seed was imported from Hungary in 1939 and was grown at Brooks the same year. Some rust developed in the plots at Ottawa in 1940, with possibly small amounts in 1941 and 1942. Later, 10 samples of seed grown at Lethbridge, Alta., in 1942, and furnished by W. D. Hay were examined, but no teliospores that could be referred with certainty to *P. carthami* were seen.

In addition, 29 samples were examined by Dr. Russell, who used a slightly different technique, but his figures may be safely compared with mine, if the former are doubled.

TABLE 2.—*Occurrence of teliospores of Puccinia carthami on seed examined at Saskatoon, Sask.*

Number of samples	Place grown	Year grown	Number of spores	
			Range	Average
5	Indian Head	1941	12–25	19.2
5	Ottawa	"	0–1	0.2
3	Lethbridge	"	0	0.0
3	Indian Head	1942	2–5	3.3
9	Saskatoon (variety plots)	"	78–233	137.1
3	Saskatoon (increase plots)	"	0–13	5.2
1	Sutherland	"	0	0.0

The results presented in Table 2 indicate that rust infection was greater in the variety plots at Saskatoon than at any other point from which seed

was examined by Russell. The infection was known to be light to moderate in these plots in September. By comparison of the spore loads recorded in the two tables, it may be inferred that the heaviest infection so far encountered occurred at Brooks, Alta. However, when this seed was planted at Ottawa in 1940, it yielded a crop but lightly infected. This fact suggests that seasonal conditions play an important part in rust development. If the appearance of this rust in Canada is due to infection from spores carried on the seed, it seems probable that the rust reached Ottawa from Brooks, and has spread from Ottawa to the other points mentioned. This is a reasonable assumption, for the Cereal Division at Ottawa has supplied, directly or indirectly, most of the lines under test in Western Canada, both to points where rust has been found and also to those where it has not been detected. Although the rust has never been reported to be destructive, it is impossible to hazard a guess concerning its importance to safflower culture until something is known of its epidemiology. Varietal differences in rust susceptibility are also to be expected.

PUCCINIA VERRUCA

A second, quite different rust occurs on safflower. It forms compact telia aggregated into relatively large groups. On safflower it has been recorded only within the boundaries of the Soviet Union (23, p. 33) in fields of the plant at Odessa, Rostov, and Omsk. On this host it originally was described as *Puccinia jaczewskii* Tropova, Journ. of Agric. Research North Caucasus **5** (22) : 211. fig. 1930 (23, p. 387). Under the name "*Puccinia centaureae*," Mourashkinsky (12) reported it as a minor disease of safflower at Omsk, where it was very prevalent on *Centaurea scabiosa* L. Later, he (13) showed by morphological studies and cross-inoculation experiments that the rust on safflower was identical with *Puccinia verruca* Thüm. (cfr. 22, **1**: 42), which is recorded on many species of Centaurea in Europe, Asia, and North Africa but does not occur in North America. Accordingly, *Puccinia jaczewskii* may be considered a synonym of *Puccinia verruca*. It is also quite probable that *Puccinia sommieriana* Sacc. (18, p. 560) described on *Centrophyllum lanatum* (= *Carthamus lanatus*) from Malta is another synonym. A specimen of *P. sommieriana* on *Kentrophyllum lanatum*, obtained at Vias, Hérault, France, by De Crozals, June, 1913. Comm. P. Hariot (Vesterg. Micr. Rar. Sel. *1719*, Herb. Arthur *F8261*) was compared with one of *P. verruca* on *Centaurea napifolia* L. (D. Sacc. Myc. Ital. *707*) and no distinguishing features were observed.

Puccinia verruca is a microcyclic species. The teliospores are narrow, smooth, usually thickened at the apex and with a long persistent pedicel. Except that the spores were less highly pigmented, they suggest those of *Puccina asteris* Duby, a microcyclic species on *Aster*. The similarity of the two species has already been noted by the Sydows (22, **1**: 43).

The close morphological resemblance between the telia of micro species and those of long-cycled heteroecious species has led to the correlation of

the respective species (8). Dietel (4, p. 492) has already pointed out the correlation of *Puccinia verruca* with its III on *Centaurea*, and now also on *Carthamus*, with *Puccinia centaureae-caricis* Tranz., which has its II, III on *Carex* and I on *Centaurea*. According to Klebahn (9, p. 516) *Puccinia centaureae-caricis* was proposed by Tranzschel in 1909 as a collective species. Klebahn reduced the *Centaurea-Carex* species of *Puccinia*, which had been described up to that time, to the rank of special forms, and united them under the above name. However, if the present rules of botanical nomenclature are followed, this rust should be known as *Puccinia arenariicola* Plowr. (Journ. Linn. Soc. **24**: 90. Aug. 20, 1887).[8] The teliospores of *Puccinia verruca* are in general narrower and less highly colored than given for any of the segregates of *P. centaureae-caricis*, but they approach most closely the dimensions given in the original description of *P. tenuistipes* Rostr., as set down by Klebahn (9, p. 518). The Sydows (22, **1**: 43) have noted that the teliospores of *P. verruca* are very variable.

AECIDIUM CARTHAMI

The third rust reported on safflower is a rare aecium, *Aecidium carthami* Dietr. (5, p. 284) collected by him at Heimar in Ehstland and reported by Rodighin (17) in the Soviet Union in the Saratov district. However, Lepik (10) recently examined the specimen issued by Dietrich (Plant. fl. balt. crypt. Cent. VIII, No. *20*) and reported that *A. carthami* is an aecium on an unidentifiable host plant. According to Curator K. Eichvald, the host may be a species of *Centaurea*, but under no circumstances is it *Carthamus tinctorius*. Even if *Aecidium carthami* was founded on an error, there is no reason to doubt that Rodighin collected an aecium on safflower. Tranzschel (23, p. 382) suggests such an aecium is possibly the result of infection by *Puccinia centaureae-caracis*. This seems a reasonable suggestion, as the correlated microcyclic species, *P. verruca*, has been shown to occur on *Carthamus*. Finally, it should be noted that Oudemans (15, **4**: 1058) is quite incorrect in listing *Aecidium carthami* as a synonym of *Puccinia carthami;* particularly, as this error appears again in the abstract of Rodighin's paper in the Review of Applied Mycology (17), where the name is written *Aecidium* [*Puccinia*] *carthami*.

SUMMARY

1. The rusts, *Puccinia carthami*, *P. verruca* and *Aecidium carthami*, have been reported on safflower.

2. *Puccinia carthami* is reported for the first time in Canada. It was collected at Morden, Man., and Saskatoon, Sask., in 1942.

3. The rust probably occurred at other points in Canada, as teliospores of the rust were found on seed samples from these points.

4. *Puccina kentrophylli*, which has been reported on some species of *Carthamus*, is not considered to be distinct from *P. carthami*. On the other

[8] Cfr. Barnhart, J. H. Bibliography. N. Am. Flora **7**: 1083. 1940.

hand, *P. carduncelli* is apparently a well-defined species, which has been reported on *Carduncellus caeruleus* and *Carthamus calvus*.

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TOMATO SEED TREATMENTS IN RELATION TO CONTROL OF ALTERNARIA SOLANI[1]

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INTRODUCTION

The area in the South devoted to growing tomato seedlings for the commercial trade has increased from an initial small acreage about 20 years ago to a total of approximately 8000 acres in 1942. The major portion of this acreage is located in southern Georgia, although important plantings are made in Florida, Mississippi, Tennessee, and Arkansas. Occasional serious losses from epiphytotics of both fungus and bacterial diseases have been one of the most serious handicaps that have confronted this expanding industry. Of the several maladies affecting tomato seedlings in the South, the one caused by *Alternaria solani* (Ell. and Mart.) Jones and Grout is the most common and, in the majority of instances, the most destructive. The losses caused by this organism result partly from damage to and a limited mortality of seedlings in the plant bed immediately after germination but, more commonly, from foliage and stem infections that cause poor stands and weakened plants in the commercial fields following transplanting. Since some part of all nontreated commercial tomato seed is known to carry a limited amount of *Alternaria solani* inoculum, the value of seed treatment as a means of control for this disease is of considerable economic interest.

REVIEW OF LITERATURE

Bichloride of mercury has long been recognized as a safe and effective chemical for the treatment of many kinds of vegetable seed, including that of the tomato. While this material is an excellent surface disinfectant, it has the disadvantage of leaving the seed open to recontamination after treatment (11). This is of particular importance where treated seeds are dried in rooms in which the air is apt to be loaded with parasitic organisms. Samson (12) and Vaughan[2] have shown that some of the organic mercury compounds have sterilizing potentialities equally as high as bichloride of mercury and, in addition, leave a residue of the active disinfectant material on the seed coat, which protects it against subsequent recontamination. Extensive tests are reported by Clayton (1, 2), Horsfall (3, 4, 5), and Moore *et al.* (9) in which these and other materials were employed as seed treatments on various vegetable seeds. Aside from that portion of the damping-

[1] Conducted as a phase of cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture and the Department of Botany, Indiana Agricultural Experiment Station; the New Jersey Agricultural Experiment Station; the Georgia Coastal Plain Experiment Station; the Georgia Agricultural Experiment Station; and the Georgia State Department of Entomology.

[2] Vaughan, E. K. The use of ethyl mercury phosphate for treating tomato seed in New Jersey.

off that may have been caused by *Alternaria solani*, no reference is made in any of these reports to the control of the disease on tomato seedlings at later stages of growth. That such treatments, however, may be beneficial in the control of this fungus in seedling fields is suggested by Miller (7), Miller and Crosier (8), and Van Haltern (15, 16).

Few if any workers have doubted the possibility of tomato seed being the source of considerable dissemination of *Alternaria solani*. Massie (6) demonstrated this as early as 1914. However, the fact that in the South[3] well-isolated fields of new soil, when planted to seed known to be thoroughly disinfected, have frequently shown infections by *Alternaria solani*, raised several questions not previously explained. Since a limited amount of the organism has been found internally in some lots of tomato seed, it has been suggested that this would account for the presence of the disease in fields of virgin soil planted with properly disinfected seed. Following some carefully conducted experiments on this phase of the seed problem, Samson (12) reports: "it appears unlikely that commercial seed may include any significant proportion internally infected with *Macrosporium solani*." Later work by Samson, Nugent, and Shenberger (14) further verifies this conclusion. In approaching the whole problem of *Alternaria solani* control, whether by seed treatment or other means, a consideration of an existing widespread dissemination of this organism in the soil throughout the plant producing area of the South is of considerable importance. Significant evidence in support of this premise is given in another report by the writers.[3] That widespread dissemination of this organism occurs in other areas is indicated by Samson and Thomas (13) who report that "*early blight* fungus is widespread, occurring naturally in all parts of Indiana, and is undoubtedly present on all forms, regardless of previous tomato crops." In order to secure additional information on the seed-treatment problem, in so far as it applies to the tomato-seedling industry in the South, a series of experiments was undertaken in 1937 and continued, with the exception of 1940, through the season of 1942. Some tests also were conducted in Indiana and New Jersey.

PROCEDURE

Commercial lots of nontreated tomato seed, known to be infected with *Alternaria solani*, were used in all experiments. All chemicals used for treatments were tested in preliminary laboratory and greenhouse tests to study their disinfectant properties and subsequent influence on germination. Effectiveness against both bacteria and fungi was determined by plating samples of treated and nontreated seed on potato-dextrose agar and incubating for 5 days at 25° C. After thus investigating many possible seed-treatment materials, 9 were chosen for critical tests under field condi-

[3] More, W. D., H. Rex Thomas, and E. K. Vaughan. Aerial dissemination of the *Alternaria solani* spores, and its possible influence upon field infection of tomato seedlings. (Unpublished manuscript.)

tions. As it became evident that certain treatments were relatively ineffective or undesirable, this number was further lowered from season to season. In 1942 only such treatments were applied as apparently gave 100 per cent surface sterilization of the seed, hence the nontreated check lots were the only seeds that carried surface contamination.

The experimental plots were located each season on land known to have been free of solanaceous crops for 10 years or more and also were well-isolated from such crops during the period of the experiments. A Latin-square design was used in all experiments, except those of 1942. The individual plot rows were 16 inches apart and 50 feet in length. The seeding rate was 20 seeds per foot. In 1942, the treatments were planted in blocks 20 feet × 22 feet, 14 rows per block, with separation alleys 20 feet wide between blocks. All treatments in this experiment were replicated 3 times. Fertilizer applications, depth of planting, and cultivation conformed to local commercial practice. Germination readings were made as soon as a commercially satisfactory stand was shown by one or more of the several treatments. Disease readings were confined to one observation, this being taken as soon as the plants reached the commercial shipping size of 6 to 10 inches high. In all cases this size was attained within 40 to 55 days after seeding. Initial leaf-spot infection was evidenced by small, dark-brown spots on the lower leaves, while stem canker appeared as small, irregular, dark-colored lesions along the main stems and leaf petioles. When the plants reached the shipping size, random samples of 50 were pulled from each replicate of the several treatments and the number of plants showing infection noted. At the same time, the total number of stem cankers per replicate was recorded. In the 1942 experiment all plants of shipping size were pulled on all plots when leaf spot first appeared.

INFLUENCE OF SEED TREATMENT ON GERMINATION

One of the most important considerations in selecting any seed-treatment material is its effect upon germination. It is particularly important to consider this in connection with Southern tomato plant production, since the seeds are planted during early spring when the weather may vary from cold rains to warm drying winds. A seed-treatment material that has a markedly retarding effect upon germination under either of these conditions is undesirable, regardless of its effectiveness in other respects. In table 1 are data showing the influence of several treatments, replicated 6 times, on seed germination when planted in sand in the greenhouse during periods of high temperature and medium humidity. Under these conditions it appears that no treatment increased stands significantly, while marked and significant decreases were shown in several instances. This was particularly true where combinations of both liquid and dust treatments were used. While the soil and air conditions during the course of these tests were admittedly not comparable to average field conditions during the spring months, the reaction

TABLE 1.—*The influence of tomato seed treatments on stands in greenhouse experiments conducted at New Brunswick, N. J., 1941*

Number	Treatments	Mean stands	
		Exp. No. 1	Exp. No. 2
1	Nontreated check	40.3	41.8
2	$HgCl_2$ (1–3000) for 10 min.	15.1	36.1
3	Hot water, 55° C. for 10 min.	43.1	44.8
4	New Improved Ceresan[a] (1–1200) for 5 min.	25.1	36.8
5	1% ethyl mercury tartrate (1–100) for 5 min.	24.8	34.1
6	Malachite green (1–2000) for 5 min.	28.1	36.1
7	New Improved Ceresan[a] (1–1200) for 5 min. + Cuprocide dust	15.0	27.8
8	Cuprocide dust[b]	26.5	39.3
9	New Improved Ceresan[a] (1–1200) for 5 min. + Vasco-4 dust	21.8	36.3
	Required difference for significance	8.2	8.6

[a] 5% ethyl mercury phosphate.
[b] Cuprous oxide.

of the various treated seed lots is of considerable value in interpreting other data collected during the course of these studies.

Field data for the same treatments in trials conducted in Georgia, New Jersey, and Indiana during 1941 are shown in table 2. Rainfall and tem-

TABLE 2.—*The influence of tomato seed treatments on stands in field experiments conducted during 1941 at Tifton, Ga., Lafayette, Ind., and Riverton, N. J.*

Number	Treatments	Mean stand per plot		
		Ga.	Ind.	N. J.
1	Nontreated check	63.5	57.2	39.2
2	$HgCl_2$ (1–3000) for 10 min.	64.2	53.6	76.1
3	Hot water, 55° C. for 10 min.	59.6	60.3	50.6
4	New Improved Ceresan (1–1200) for 5 min.	63.8	45.2	68.6
5	1% ethyl mercury tartrate (1–100) for 5 min.	64.3	50.7	69.3
6	Malachite green (1–2000) for 5 min.	61.9	59.7	84.5
7	New Improved Ceresan (1–1200) for 5 min. plus Cuprocide dust	63.8	42.7	47.0
8	Cuprocide dust	55.7	57.4	80.2
9	New Improved Ceresan (1–1200) for 5 min. plus Vasco-4 dust	60.0	48.7	76.6
	Required difference for significance	Not significant[a]	5.3	12.0

[a] F = 0.41. Required value at 5% = 2.52.

peratures in Georgia and Indiana were sufficient for germination, whereas in New Jersey, rain was heavy and temperatures comparatively low. Accordingly, only a few treatments suppressed germination significantly below the nontreated check, and several appreciably increased stands. Other germination data of a similar nature collected in seasons of light rainfall and moderate temperature are given in tables 4, 5, and 6. In the New Jersey experiment of 1941 the seed lots were planted in soils that were fairly cold

and wet. From the data presented it is apparent that under those conditions seed treatments increased germination significantly in several instances. That such reactions may be associated with cold, rainy seasons is suggested by additional stand data (table 3) taken in Georgia in 1937, when the soil was cool and moist during the period of germination.

TABLE 3.—*The influence of tomato seed treatments on stands and on the control of Alternaria solani leaf-spot infection at Tifton, Ga., in 1937*

Number	Treatments	Mean stand per plot	Mean number leaf spots per plot sample[a]
1	Nontreated check	47.3	57.3
2	Acidulated $HgCl_2$ (1–3000) for 5 min.	60.4	53.3
3	$HgCl_2$ (1–3000) for 5 min.	62.3	41.1
4	Formaldehyde (1–200 at 120° F.) for 5 min.	50.8	53.3
5	Hot water, 55° C. for 10 min.	55.2	50.7
6	Sodium hypochlorite (1–10) for 5 min.	50.9	43.4
7	Semesan Jr. (1–160) for 5 min.	77.2	49.7
8	New Improved Ceresan (1–800) for 5 min.	76.4	42.1
9	5% ethyl mercury iodide (1–800) for 5 min.	61.6	46.0
10	1% ethyl mercury tartrate (1–160) for 5 min.	72.7	40.3
	Required difference for significance	16.6	Not significant[b]

[a] No stem cankers.
[b] F = 1.77. Required value at 5% = 2.00.

TABLE 4.—*The influence of tomato seed treatment on stands and on the control of Alternaria solani leaf-spot and stem-canker infections at Tifton, Ga., in 1938*

Number	Treatments	Mean stand per plot	Mean number leaf spots per plot sample	Mean number stem cankers per plot sample
1	Untreated check	117.0	67.3	36.0
2	$HgCl_2$ (1–3000) for 10 min.	72.5	82.8	21.4
3	Hot water, 55° C. for 10 min.	101.8	80.9	33.1
4	New Improved Ceresan (1–1200) for 5 min.	75.3	82.0	25.2
5	1% ethyl mercury tartrate (1–100) for 5 min.	100.2	87.1	27.7
6	Malachite green (1–200) for 5 min.	76.1	72.4	34.0
7	New Improved Ceresan (1–1200) for 5 min. plus Cuprocide dust	83.1	79.8	29.6
8	Cuprocide dust	97.6	77.9	26.9
9	New Improved Ceresan (1–1200) for 5 min. plus Vasco-4 dust	56.0	78.0	17.5
	Required difference for significance	4.0	Not significant[a]	Not significant[b]

[a] F = 1.3. Required value at 5% = 2.07.
[b] F = 1.0. Required value at 5% = 2.07.

INFLUENCE OF SEED TREATMENT ON LEAF SPOT AND STEM CANKER

Since the development of *Alternaria solani* in either the leaf-spot or the stem-canker stage is markedly limited by the prevailing humidity (10),

wide variation in amount of disease may be expected from one season to another. This was true in the present investigation, where the disease was entirely absent in one season, was present in only the leaf spot stage in two, and caused both leaf spot and stem canker in the others. Due to such variation in seasonal disease development, it was necessary to continue the work

TABLE 5.—*The influence of tomato seed treatment on stands and on the control of Alternaria solani stem-canker infection at Lafayette, Ind., in 1938*

Number	Treatments	Mean stand per plot	Mean number stem cankers per plot sample
1	Nontreated check	49.2	2.1
2	$HgCl_2$ (1–3000) for 10 min.	45.0	2.0
3	Hot water, 55° C. for 10 min.	46.6	0.8
4	New Improved Ceresan (1–1200) for 5 min.	51.0	0.9
5	1% ethyl mercury tartrate (1–100) for 5 min.	43.8	1.4
6	Malachite green (1–2000) for 5 min.	41.6	1.8
7	New Improved Ceresan (1–1200) for 5 min. + Cuprocide dust	55.8	1.4
8	Cuprocide dust	52.8	1.9
9	New Improved Ceresan (1–1200) for 5 min. + Vasco-4 dust	52.0	1.9
	Required difference for significance	6.0	Not significant[a]

[a] F = 1.3. Required value at 5% = 1.99.

over a period of 6 years in order to collect sufficient data to warrant definite conclusions as to the value of the seed treatments for control of *Alternaria solani*. From the leaf-spot and stem-canker data presented in tables 3, 4, 5, 6, and 7, it is apparent that in only one instance (1939), was infection by

TABLE 6.—*The influence of tomato seed treatment on stand and on the control of Alternaria solani leaf spot at Tifton, Ga., in 1939*

Number	Treatments	Mean stand per plot	Mean number leaf spots per plot sample
1	$HgCl_2$ (1–3000) for 10 min.	242.4	37.2
2	New Improved Ceresan (1–1200) for 5 min.	238.0	31.6
3	New Improved Ceresan (1–1200) for 5 min. + Cuprocide dust	187.0	22.4
4	New Improved Ceresan (1–1200) for 5 min. + Vasco-4 dust	243.0	30.4
5	Check	346.0	40.4
	Required difference for significance	8.3	11.0

Alternaria solani reduced significantly by seed treatments. In this instance (treatment 3, table 6) germination was delayed. Consequently, the less advanced age and maturity of the plants appeared to be the determining factor rather than the surface sterilization of the seed. This relationship of seedling age to infection by *Alternaria solani* has been demonstrated

TABLE 7.—*The influence of tomato seed treatments on the control of Alternaria solani leaf-spot infection at Tifton, Ga., in 1942*

Number	Treatments	Mean number leaf spots per plot sample
1	Nontreated check	77.4
2	$HgCl_2$ (1–3000) for 10 min.	80.6
3	New Improved Ceresan (1–1200) for 5 min.	81.5
4	Malachite green (1–2000) for 5 min.	80.6
5	New Improved Ceresan (1–1200) plus Cuprocide dust	84.1
		Not significant[a]

[a] F = 0.22. Required value at 5% = 2.82.

under southern field conditions in other experiments by two of the writers.[4] In nearly all instances, the amount of infection increased with the increasing age of the seedlings.

DISCUSSION

The influence of tomato-seed treatments on germination and subsequent stands apparently is determined by prevailing weather conditions. During periods of medium to high temperature and moderate to low rainfall the liquid organic mercury treatments and the copper dust treatments have a tendency to depress germination to a point somewhat lower than that of the nontreated seed. During periods of cool wet weather, however, significant increases in stands occurred after both the liquid and the dust treatments. While there were instances where field stands were reduced by certain treatments, the reductions in most cases were not of appreciable importance under the conditions encountered by the writers. Since significant increases were had during cool wet seasons, the practice of seed treatment, if only for protection against damping-off, is highly advisable.

The failure of seed treatments to control *Alternaria solani* on tomato seedlings in the field, under the conditions of these experiments, is due apparently to a wide occurrence of the organism in the soil throughout the plant growing area and not to a lack of thorough surface sterilization of the seed. With a combined annual seedling and market tomato planting of 12,000 to 15,000 acres in southern Georgia, there is ample opportunity for wide distribution of inoculum. Even though there are available several materials that will effectively disinfect tomato seed, there is likely to be a sufficient amount of inoculum already present in all commercial fields, whether old or new, to insure heavy infection under proper weather conditions.[5] This apparently precludes the possibility of a general control of this disease by means of seed treatment alone. While the results of these experiments are not in agreement with the findings of some other investigators (7, 8, 14, 15),

[4] Moore, W. D., and H. Rex Thomas. Some cultural practices that influence the development of *Alternaria solani*. (Unpublished manuscript.)

[5] See footnote 3.

the differences in results appear to be due to differences in circumstances or prevailing conditions rather than in crop technique. In areas outside of the tomato-growing district of Georgia the present data may not apply. Within the restricted commercial areas, however, the above findings have been further verified as to commercial practice from the plant certification records of the Georgia Department of Entomology. From hundreds of cases over a period of 3 years, wherein records were made of seed infection, crop history of the fields, spraying, and disease incidence, no consistent differences were observed in *Alternaria solani* infection between fields planted with treated seed and those planted to nontreated seed. The lack of control of this particular organism in these experiments, however, does not minimize the importance of seed treatment to the tomato-plant industry. The necessity for control of *bacterial leaf spot* and protection against damping-off fully justifies the practice.

SUMMARY

Both liquid and dust seed treatments have a tendency to retard tomato seed germination during periods of high temperatures and medium to low rainfall. During the two seasons of cool wet weather experienced in the course of this research, final germination was improved significantly by the organic mercury treatments. Bichloride of mercury also gave significant improvement in one of these seasons, and malachite green and Cuprocide dust showed effectiveness during the one season in which they were included.

With the exception of one treatment (Ceresan plus Cuprocide) in 1939, seed treatments did not reduce *Alternaria solani* leaf spot or stem canker significantly in experiments conducted in four different years. Since the treatment mentioned caused no reduction in infection when tested in other years, it appears that the treatments here described are not likely to greatly reduce the amount of *Alternaria solani* infection in the tomato-plant-growing sections of southern Georgia.

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OBSERVATIONS ON THE BACTERIAL CROWN, STEM, AND BUD ROT OF DELPHINIUM

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The stem and bud rot caused by *Erwinia phytophthora* (Appel) Comm. S. A. B. has been destructive for several years in commercial and garden plantings of rocket larkspur (*Delphinium ajacis* L.) in California (1). Also, it frequently has caused serious losses in plantings of perennial delphinimum, producing a crown rot of the type illustrated by Blodgett (2). On either of these hosts the disease may under favorable environmental conditions assume epidemic proportions. Opportunity has been afforded during the last 3 years for observations of conditions favoring this disease. Because production of larkspur seed has been increased to supply medicinal needs of the armed forces, these observations are presented at this time.

The symptoms of this disease have been adequately described for perennial delphinium (2, 7) and larkspur (1, 4) and are not, therefore, discussed in this paper.

Excessively wet soil greatly increases the injury to infected plants and facilitates spread of the bacteria, as observations in June, 1942, in Santa Barbara County clearly showed. In one seed field of rocket larkspur, in which the lower third of the planting had been watered unintentionally by irrigation waste from an adjoining field at the time the flower buds were forming, 20 per cent of the plants in the wet area were infected (sample of 400 counted). No infected plants were found in the rest of the planting in which the soil was dry and hard. In another such field, irrigated a few days before, the lower ends of the rows had been flooded and were much wetter than the rest of the field. In the flooded area 59.5 per cent of the plants were infected (sample of 242 counted) and in the irrigated, but relatively drier portion, 7.4 per cent were infected (sample of 446 counted). In a third field of larkspur a low area, adjacent to an irrigation standpipe, had no plants in the center, nearly all plants diseased at the margin, and only an occasional diseased plant in the surrounding field (Fig. 1). These observataions agree with those on blackleg of potato caused by the same organism (6).

Similarly, the incidence of bacterial crown rot in perennial-delphinium plantings is greatly increased by excessively wet conditions. An experimental planting that had been watered by overhead sprinklers, when observed in West Los Angeles in June, 1940, was so severely injured that several types were totally destroyed. As soon as such watering was discontinued the losses sharply declined. In the same year a nearby commercial planting of perennial delphinium suffered similar losses, being heavily irri-

[1] The writer is grateful to K. F. Baker for direction, advice, and kind criticism received in the preparation of the manuscript.

gated in furrows at rather frequent intervals. Only mild losses have been sustained in both the experimental and commercial plantings in the 2 years since, probably because the irrigation has been kept to an absolute minimum, particularly after elongation of the flower spike begins. Observations during the same period in other nearby plantings where irrigation was not regulated and where serious losses from this disease occurred, indicated that these seasons were favorable to the disease. In Southern California it is usually necessary to irrigate perennial delphinium in order to obtain maximum growth. It is possible to irrigate with only negligible losses by making the furrows some distance from the rows and having the crowns of the plants on low ridges.

FIG. 1. Part of a field planting of rocket larkspur that had been flooded from a nearby irrigation standpipe, showing the increased severity of the disease resulting from excess water. Photographed June 16, 1942.

The cracking of the stems of perennial delphinium at or near the soil level appears particularly to predispose them to infection. Such cracks are illustrated by Blodgett (2). Since these cracks appear at the time the flower spike begins to elongate, this developmental stage may be regarded as the critical period for this disease. This is also true for rocket larkspur (1). Observations indicate that fluctuating conditions of soil moisture and perhaps temperature aggravate this apparently natural cracking. Under wet, poorly aerated conditions such cracks do not heal, and may serve as open avenues of infection. These observations are in general agreement with those on the healing of cut seed-pieces in relation to potato blackleg (6). Leonian (7) states that young delphiniums may be planted successfully in the spots where old, diseased plants died. Since stem cracking occurs in late

stages of growth, the escape of young plants is perhaps to be expected. However, such replanting would hardly seem an advisable garden practice.

The disease has been consistently most damaging in the summer months, and is regarded by investigators (2, 5, 7) and growers as favored by warm weather. While this seems to be a correct generalization, it should be recorded that losses can be severe during winter months. In February, 1941, after several months of heavy rain, practically all of the abundant volunteer larkspur plants in a field in Santa Barbara County were found infected and many killed.

Observations show that soil carry-over may greatly increase the incidence of the disease. In 1942, a strip 45 yards wide along one end of a field of

FIG. 2. Field planting of rocket larkspur variety Super Majestic Rose, showing in the foreground the area which had been planted to larkspur the previous season and in the background the area not previously planted to this crop. Photographed June 16, 1942.

rocket larkspur extended into an area in which the same plant had been grown the previous year. The plants occurring in this strip showed a high incidence of disease, the line of demarcation separating the diseased plants from the healthy coinciding closely with the margin of the overlap. In the overlapped planting, Larkspur var. Blue Spire showed 58.0 per cent, diseased plants, Lustrous Carmine in one block 54.0 per cent and in another 92.8 per cent, Los Angeles 71.7 per cent, and Super Majestic Rose 93.6 per cent (Fig. 2). In all varieties the plants counted as healthy were badly wind beaten or broken over, so that from the standpoint of seed production the area was practically a total loss. In the clean planting there was little or no evidence of the disease. The causal organism apparently is able to live over in soil with a high degree of infectivity from one planting cycle to the next, a period of at least 6 months. From the standpoint of field control,

therefore, it would be a wise precaution to practice a crop rotation of at least 1 year and preferably 2. However, it should be pointed out that in some cases the disease incidence may be high in plantings where delphiniums have never previously been grown. In the experimental planting of perennial delphiniums mentioned above, the young plants were grown in pasteurized soil and transplanted into a field which had not been cultivated for at least 10 years and which apparently had never before been planted to delphiniums. All plants of a number of varieties were killed. Similar experiences are reported by Blodgett (2).

The hot-water treatment of rocket larkspur seed that has been recommended (1) for this disease might reasonably be effective through reduction of inoculum potential, but evidence on this point in commercial seed fields is somewhat conflicting. It is expected that it would be more effective on soils of low than of high inoculum potential.

The observations reported above indicate that two variable factors, soil moisture and inoculum potential, may determine the incidence of this disease in the field during the summer months. Under conditions of low inoculum potential no disease or only slight losses occur under conditions of low soil moisture but severe losses may occur, though tardily, if the soil be waterlogged; this situation is illustrated in figure 1. Under conditions of high inoculum potential, prompt, heavy losses result if the soil receives more than a minimum of water, but the same amount of water may be applied safely to soil of low inoculum potential; this condition is illustrated in figure 2. Soils of heavy inoculum potential have been successfully utilized for perennial delphinium by applying a minimum of water at a distance from the crowns. It is also in the interest of maintaining a low inoculum potential in the soil to avoid overirrigation.

Because commercial seed plantings in California are made before or during the rainy season it is not always possible to control the factor of soil moisture, and crop rotation must be practiced. In California home gardens it is customary to plant seedlings of rocket larkspur rather than seed and planting may, therefore, be delayed until the moisture factor may be effectively controlled. Rotation, while desirable, may not in such cases be imperative. Seedlings of perennial delphinium transplanted to infested soil in early spring may be successfully grown through the first season by careful attention to watering; they are generally not grown for a second season in California because of the high percentage of aster yellows that appears. Application of Bordeaux mixture (4, 9) and mercuric chloride (9) around the crowns of delphiniums has been suggested for control of this disease but has not been tested in California.

With rocket larkspur the black stem streaking, characteristic of diseased plants, often does not extend down to soil level, suggesting transmission of the disease through some aerial agency. Seed transmission and subsequent splashing of bacterial exudate from plant to plant during foggy or wet windy weather are possible explanations, but insect vectors may be involved

(1). Slugs have also been suggested (9) as carriers of the bacteria. Trogoderma beetles were found associated with the bacterial exudate from stems of perennial delphinium in Idaho (2). Greenhouse tests have shown that when mature parts of rocket larkspur plants are sprayed with a bacterial suspension, they do not develop symptoms, infections occurring only in the growing apices and developing buds after an incubation period of about 1 week. These results agree with the observations of Chester (3) on the nonoccurrence of stomatal infection. Plants hypodermically inoculated at the base, however, may be totally destroyed within 30 hours.

Delphiniums have been noted to differ considerably in susceptibility to the bacterial stem and bud rot. Varieties of rocket larkspur that have been particularly susceptible in field plantings are: The Dazzler, Miss California, Lilac King, Stock Flowered Dark Blue, Blue Bell, Super Majestic Rose, Lustrous Carmine, and Los Angeles. Of the perennial delphiniums,[2] the Belladonna group, represented by the varieties Belladonna, Bellamosum, and Lamartinii, is highly susceptible under field conditions, the infection often occurring high on the stem. Of the *Delphinium elatum* L. group, the large candelabrum types listed as Pacific Giant Hybrids, the English (Wrexham) hollyhock strain, and the Blackmore and Langdon strains, are all susceptible. The Grandiflorum group, including the varieties listed as *D. chinensis* Hort. and the spurless types listed in the trade as *D. cinereum* Hort. and *D. tatsienense* Hort., all appear under field conditions to be highly resistant. Of California native delphiniums, *D. cardinale* Hook., *D. hesperium* Gray, *D. parryi* Gray, and *D. scopulorum* var. *glaucum* Gray, have been noted as being very susceptible under garden conditions. Varietal differences in susceptibility of perennial delphinium were also noted by Blodgett (2) but details were not reported.

SUMMARY

The bacterial crown rot of perennial delphinium and the stem and bud rot of rocket larkspur, caused by *Erwinia phytophthora*, are strongly favored by excessive soil moisture. It is possible to greatly reduce losses from these diseases by application of a minimum of water by means of furrows at a distance from the rows, and by planting on low ridges. Particular care in irrigation is necessary after the flower spikes begin to elongate.

The bacteria are able to live over in soil in a high degree of infectivity at least from one planting cycle to the next and crop rotation is, therefore, advisable in the commercial production of seed and, where possible, in ornamental plantings. The disease may become destructive on soils of low inoculum potential if the soil is saturated with water; in the interest of maintaining a state of low infectivity in soil excessive irrigation should be avoided.

[2] Information on the perennial types was kindly supplied by G. A. L. Mehlquist. The taxonomic arrangement followed is that of Mehlquist, Blodgett, and Bruscia (8).

While the disease definitely is worse on both hosts in the summer months it may become destructive during the winter.

The time of flower-spike elongation is the critical period for this disease in the life of both types of delphinium. This seems to be correlated with the appearance of deep cracks in the stem bases.

Varietal differences in susceptibility to this disease have been noted in both rocket larkspur and perennial delphinium.

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ARMILLARIA ROOT ROT OF FRUIT TREES IN THE EASTERN UNITED STATES

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When the writer began work on the problem of root rot of deciduous fruit trees in 1931, Armillaria root rot was included in the project. The world-wide distribution of this disease and its importance in the western part of this country made it seem desirable to ascertain to what extent it is responsible for the loss of peach trees in the eastern part of this country. Armillaria was known to be present in the Sand-hill region of North Carolina and adjacent States, where heavy losses from root diseases of peaches occurred.

This paper reports a study of Armillaria root rot, giving the results of field surveys on distribution and severity, also results of inoculation experiments with peaches, apples, and pears, carried out in Virginia, Maryland, and replanting experiments with peaches in North Carolina.

REVIEW OF LITERATURE

Very early in the history of plant pathology, Armillaria was recognized by Hartig (4) in Germany as causing a root disease. Since that time it has been reported from many regions widely separated over the world, and many papers have appeared dealing with the disease. Wallace (8) states that close to 200 host plants for this pathogen have been recorded in the literature.

The extent of the literature on this disease is shown by Reitsma (6) who lists 494 citations, and more have appeared since the publication of his paper in 1932.

Various workers on this disease have reported widely divergent views on the virulence of its pathogenicity. Many workers have considered Armillaria an active parasite. Hendrickson (5) states, with reference to prunes, that the vigor of an individual tree is without effect on resistance to the disease.

Some of the workers, particularly the more recent ones, hold a different view concerning the parasitism of Armillaria. Reitsma (6) states: "It is a facultative parasite with properties of a perthophyte."

Day (3) states that Armillaria is a secondary parasite on the oak, spruce, and fir, with such predisposing factors as mildew on the oak and drought and sun-scorch on the fir and spruce. He reports a Corsican pine affected by the fungus but showing no evidence of serious injury so long as it occupied a dominant forest position. After losing its dominance by growth of younger trees, this tree was rapidly invaded and killed by Armillaria.

Butler (1) reports that collar crack of tea bush in Africa, caused by Armillaria, results in serious loss only after the soil becomes infertile from

erosion. Dade (2) reports that this disease on the cacao in Africa is epidemic only on soil of excessively high moisture content.

This diversity of opinion concerning the pathogenicity of this fungus may be due to an extreme variation in strains of the organism or it may be due to differences in environmental conditions favoring or retarding the resistance of the host. Although it is not possible to separate the host and parasite in their relationships to environment, the observations and experiments reported in this paper indicate that unfavorable environment for the peach as host is especially important in inducing susceptibility.

SYMPTOMS OF ARMILLARIA ON PEACH AND OTHER STONE-FRUIT TREES

The leaves of trees affected by the Armillaria disease roll along the midrib, and have a wilted appearance. Because the internodes are much shortened and growth is greatly retarded, the trees have a stunted appearance. In the later stages the limbs on the same side as the affected roots almost cease growth, are a light green, or may die.

This disease, like other root disturbances, cannot be positively identified by the visible symptoms on the above-ground parts, for the symptoms on the foliage may be similar to those produced by other root troubles, such as root winter injury and collar winter injury, or root suffocation.

The most characteristic symptom on stone-fruit trees is the white to cream-colored mycelial growth in the cambium of the affected roots or root crown. On margins of the diseased area the fungus may spread out in the cambium in distinctive fan-shape pattern. The fungus also advances into the wood of affected roots, producing a decay that becomes progressively lighter, eventually turning to a very light tan or cream color.

Another symptom of the disease is the presence of black rhizomorphs adhering to the surface of affected roots. These rhizomorphs advance through the soil and thus spread the disease from root to root and tree to tree. Of course, one of the most definite diagnostic characters of the disease is the fruiting body of the fungus, which sometimes forms about the collar of affected trees or on shallow roots. In culture, the fungus is readily distinguished from most other fungi by the formation of honey-colored rhizomorph-like structures permeating the agar.

ORCHARD SURVEYS ON THE PREVALENCE OF THE DISEASE ON POME AND STONE-FRUIT TREES

From 1931 to 1942, in connection with other root-rot studies, surveys on the incidence of this root rot have been made in Virginia, Maryland, North Carolina, and other eastern and central States. Since Armillaria occurs as a saprophyte in the deciduous forests in these regions and is known to rot the roots of a wide variety of cultivated plants, including the apple and the peach, an inspection to determine its presence and pathogenicity on apple and peach trees was made at every opportunity. The results of these obser-

vations were different from what might have been expected, judging from the literature on the general distribution of this disease.

Armillaria on Apple Trees

In the Hood River Valley, Oregon, Armillaria has been observed by the writer, growing on the collar of apple trees affected with the sunscald type of winter injury on the trunk, but casual observation indicated that it was not active as a parasite on the roots of uninjured apple trees. In the eastern part of the United States surveys for various root disturbances have been made in many of the fruit districts. In only one case was Armillaria found growing on apple roots. This tree, which was located in Virginia, was growing under adverse conditions because of wet, poorly drained soil.

Armillaria on Cherry and Peach Trees

Surveys have been made of the cherry orchards in northwestern Pennsylvania, but no Armillaria root rot was observed. In one case a cherry tree that had been planted on newly-cleared land in Maryland was killed by Armillaria root rot. The observations on cherry trees, though limited, indicate that Armillaria root rot is not of frequent occurrence or serious in the eastern part of this country.

Observations on root diseases of the peach have been made in a number of peach orchards in Georgia and in the Shenandoah-Cumberland region of Pennsylvania, Maryland, and Virginia, but Armillaria root rot was not found.

In the Coastal Plain of Maryland, Armillaria root rot was occasionally found. Annual observations on an affected tree have been made during the past 8 years and are here recorded. In 1933, a 3-year-old peach tree replant in a 6-year-old peach orchard, growing in sandy soil in the Coastal Plain of Maryland was found to be diseased with Armillaria and was removed. The next year an adjacent tree, also a replant, showed disease symptoms and an examination, together with isolations, showed the presence of Armillaria, several roots being affected at that time. This tree was allowed to stand and examinations have been made each year since then to find out how rapidly the disease spread to sound roots and the time required to kill the tree. Although the disease has been gradually advancing, the tree has been able to make new roots above the diseased ones and was still alive in 1942. In 1941, the tree ceased active growth but the poor vigor was at least partly due to borers. In spite of the massive invasion and, presumably, an abundance of inoculum in the soil, the spread of the disease to other roots has not been rapid. The fact that a diseased tree remained alive at least 8 years after it was known to be infected and that the adjacent trees have not given indication of being affected, indicates that the fungus has not invaded rapidly and that conditions have not been favorable for spread to or infection of adjacent trees. These two trees were growing in a slight depression where there was some evidence of slow drainage after heavy precipitation.

In the sand-hill section of North Carolina, Armillaria was frequently found on peach trees. In this region most of the peach orchards were set on land newly cleared of partly deciduous forest, thus providing favorable conditions for an abundant growth of the pathogen in the soil. Certain adverse host conditions, however, existed. The soil is very light and sandy and is probably low in humus and mineral plant foods. Also, climatic conditions have tended toward temperature and moisture extremes. An injury about the collar, seemingly winter injury, was prevalent. Furthermore, nematode injury contributed to the complexity of the situation. It was difficult therefore to determine to what extent Armillaria was the primary cause of the death of affected trees.

INOCULATION EXPERIMENTS

Inoculation work was begun in 1933 and has been carried on for 9 years, using 1- to 5-year-old nursery trees of apple, pear and peach growing at Arlington Experiment Farm, Arlington, Virginia, at the Bureau of Plant Industry Station, Beltsville, Md., and at Fruitland Orchard, Hamlet, North Carolina. The inoculum used at first was a pure culture of the fungus, obtained from apricot root from The Dalles, Oregon, and grown on peach twigs about 2 inches long and 1½ inches in diameter. It was expected that the use of such large pieces would favor production of rhizomorphs, which Thomas (7) states are necessary for infection. In the later inoculation work the inoculum was grown on pieces of apple limb an inch or more in diameter and about 2 inches long, that had been placed in jars with wheat bran, then sterilized and subsequently inoculated. After several months, when the wood was well covered with the fungus, inoculation was made by scraping the outer bark of the root with a trowel, laying a piece of the inoculum on the root, and replacing the soil. This method of inoculation was suggested by Harold Thomas and Donald Bliss of the University of California.

In 1934, at Arlington Experiment Farm, periodic monthly inoculations were made on apple, pear, cherry, plum, and peach trees that had been growing in the nursery for 3 years. Many of the peach trees used in later inoculation work were of bearing age, but were growing in close nursery formation.

In 1938, an experimental plot of peach trees was set at Hamlet, N. C., with 1-year-old peach trees of a known root and top combination, the roots being seedlings of known commercial varieties. These trees were set 4 feet apart in rows 8 feet wide in a plot in a 10-year-old peach orchard where the trees had died. Armillaria was present on some of the old peach trees still standing only a short distance from the experimental plot. The cause of the death of old trees in that part of the orchard was not definitely determined. The conditions obtaining there were probably typical of many other orchards where dead trees occurred. The next year after the trees were planted the roots were inoculated in July with a strain of the fungus from North Caro-

lina. The next year inoculation was made on the opposite side of the main root.

The results of all these inoculations in North Carolina, Virginia, and Maryland, were, in the main, negative. In a few cases, lesions as much as 10 mm. in diameter were produced at the point of inoculation on cherry or peach-tree roots; however, these lesions soon healed over. The results of all apple and pear inoculatons were negative. At the Hamlet plot, some trees died the first year because of late planting and hot weather. Some trees died each year but there was no evidence that any root-rot pathogen killed the trees.

DISCUSSION OF RESULTS

The fact that the isolates used for inoculum were taken from a variety of sources, including widely separated geographical regions, suggests that the absence of infection was due to host resistance rather than failure to obtain normally virulent strains of inoculum. These negative inoculation results agree with field observations in eastern United States in indicating that the disease occurs rarely and that the edaphic conditions where most of these observations were made were not suitable for host infection. Where it does occur there is evidence that the host has been weakened by an unfavorable environment.

The references in the literature to Armillaria as causing a serious root disease of cultivated plants are much more likely to emanate from a region of low precipitation, as South Africa or West Africa or California, than from a more humid region.

The evidence given above indicates that the comparative absence of the disease in the eastern United States and its prevalence in the West Coast regions are due to host reaction to physical environment. That is, the environmental conditions in the West Coast regions are favorable to host susceptibility, and in the eastern regions conditions are, in the main, unfavorable to susceptibility. It may be that the xeric conditions of the West favor host susceptibility to this pathogen.

SUMMARY

Surveys in many of the fruit regions in the eastern and central parts of the United States resulted in occasionally locating stone-fruit trees attacked by Armillaria. The pathogen has been isolated from peach roots from the Sand-hill region of North Carolina, but no evidence has been obtained showing whether Armillaria is the initial cause of the decline of the trees or whether the fungus follows some disorder caused by adverse environment.

Observations for 8 consecutive years on a diseased peach tree growing in the Coastal Plain of Maryland showed a slow advance of Armillaria in the affected tree, but no indication of spread to the surrounding trees.

At monthly intervals for 2 years inoculations were made on young pome and stone-fruit trees. These usually gave negative results, but in some in-

stances small lesions were formed. These healed by the next year. Inoculation of replants in an old peach orchard in North Carolina in which Armillaria was present also failed to produce the disease. Natural infection has not occurred on these test trees.

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INFLUENCE OF CONTACT PERIOD ON THE PASSAGE OF VIRUSES FROM CION TO STOCK IN TURKISH TOBACCO

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INTRODUCTION

Extensive evidence indicates that there are distinct differences between viruses in their relationships to the tissues of the plants in which they occur. Those viruses that cause generalized yellowing, leaf curling, leaf rolling, etc., without mottling, appear as a rule to be more or less limited to the phloem of the invaded plants and the symptoms produced are typical of disturbances arising in the phloem. Those viruses that cause mottling and various types of local lesions occur in relatively high concentrations in the parenchyma and produce symptoms typical of disturbances arising in the parenchyma. However, it is evident that most viruses of this latter type occur also to some extent in the phloem, although their concentration in this tissue may be relatively low as compared to that in the parenchyma.

It would be expected that viruses of these two groups would exhibit differences in their ability to pass from an infected cion or bud to a healthy stock through a graft union. Obviously, if a virus is limited to the phloem it cannot pass from a diseased cion to a healthy stock until a phloem bridge is formed across the graft union; whereas a virus that increases and moves in parenchyma should be able to pass the graft union as soon as a parenchymatous bridge becomes available, perhaps considerably before there is differentiation of phloem in the tissue uniting stock and cion.

Evidence that may be interpreted as support for this hypothesis was obtained by Kunkel[1] who showed that a shorter contact period was required for the transmission of peach mosaic than for that of peach yellows, little-peach, and peach rosette. No such studies, however, are available on herbaceous plants, possibly because of the difficulty of selecting viruses with the desired tissue relationships and at the same time not transmissible by manipulation necessary in making grafts. Recently, two viruses have been found, one a strain of a ring-spot virus and the other a strain of cucumber-mosaic virus that are transmissible to Turkish tobacco by juice inoculation, only with considerable difficulty. These two viruses have been compared with the virus of curly top with respect to the readiness with which they will pass from an infected cion to a healthy stock. The results of these experiments are presented in this paper.

MATERIALS AND METHODS

The strain of curly-top virus used in these experiments was one that is relatively severe on sugar beet and causes severe symptoms on Turkish

[1] Kunkel, L. O. Contact periods in graft transmission of peach viruses. Phytopath. 28: 491–497. 1938.

tobacco. The virus has been carried in sugar beet and tobacco for several years.

The ring-spot virus came originally from field tomatoes but has been held in Turkish tobacco for more than two years. Repeated attempts to transmit this virus to Turkish tobacco and tomato by juice inoculation have resulted in only a few cases of infection. In a few instances limited numbers of local lesions were produced on Turkish tobacco but in most cases neither local lesions nor systemic infection resulted from juice inoculation. Systemic infection did not always follow the production of local lesions. Plants that are systemically infected produce rings and spots of necrotic tissue on the younger leaves as primary symptoms after which the plant recovers to the extent that no obvious symptoms are evident on the subsequent growth.

The strain of cucumber-mosaic virus used came from sugar beet on which it causes a severe form of mottling. It is transmissible to Turkish tobacco by juice inoculation but only very small percentages of the inoculated plants have become infected.

Four lots of diseased Turkish tobacco plants were prepared as sources of diseased cions used to make the graft inoculations. Lot 1 was infected by curly-top virus; lot 2 by ring-spot virus; lot 3 by curly-top virus plus ring-spot virus; and lot 4 by curly-top virus plus ring-spot virus plus cucumber-mosaic virus. Healthy Turkish tobacco plants were grown to a height of about 2 feet. The stems were cut back to a height of about 10 inches. Cions from the 4 lots of diseased plants were attached at the tops of the stems, the area of contact between cion and stock being a slanting surface somewhat more than an inch in length. Cions were removed after the alloted period of contact. The contact periods between cion and stock varied from 1 to 14 days with a 1-day interval for the first 6 days and a 2-day interval thereafter.

EXPERIMENTAL RESULTS

The detail results of this series of tests are shown in table 1. It will be noted that no curly-top infection occurred in plants in which the union with diseased cions was less than 5 days. In general there was an increase in percentage infection as the time of contact with diseased cions was increased. Infections by the viruses of ring spot and cucumber mosaic began with the plants having a 2-day contact period with infected cions, and increased rapidly as the period of contact was increased. The fact that no infection took place on any of the plants in which the contact period was 1 day indicates that there was no infection resulting from handling the plants during the process of making the grafts.

Infection by curly-top virus and ring-spot virus from cions that carried both viruses closely parallels that obtained where the two viruses were in separate cions. This is true also from the cions that had all 3 viruses. There is some evidence, however, from the results obtained from the cions containing all 3 viruses that the ring-spot virus moves more rapidly

into the healthy stock than does the cucumber-mosaic virus. This is evident from the figures showing infection. Analysis of individual plant records showed that 23 of the plants inoculated with the triple virus combination were infected by only one virus. Of these, 19 were infected by ring-spot virus and only 4 by cucumber-mosaic virus. Further tests in which the viruses of ring spot and cucumber mosaic were combined in the same cion have given similar results. Of 123 plants infected by only one virus, 102 were infected by ring spot and 21 by cucumber mosaic. There was no instance of infection by curly top alone where this virus was in combination with one or both of the other viruses.

TABLE 1.—*Influence of period of contact on passage of viruses from diseased cion to healthy stock in Turkish tobacco*

Period of contact between cion and healthy stock	Plants infected[a] from cions carrying the indicated viruses and combination of viruses						
	Curly-top virus	Ring-spot virus	Curly-top virus plus ring-spot virus		Curley-top virus plus ring-spot virus plus cucumber-mosaic virus		
	Curly top	Ring spot	Curly top	Ring spot	Curly top	Ring spot	Cucumber mosaic
Days	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	0	0	0	0	0	0	0
2	0	3	0	6	0	2	2
3	0	9	0	8	0	10	6
4	0	13	0	11	0	15	10
5	2	15	1	12	0	15	10
6	5	15	4	13	2	15	15
8	7	15	6	15	6	15	14
10	9	15	10	15	7	15	15
12	11	15	12	15	11	15	15
14	14	15	15	15	14	15	15

[a] Figures represent the number of plants infected out of 15 inoculated in each instance.

DISCUSSION AND CONCLUSIONS

The results of this series of graft inoculations shows clearly that the virus of ring spot and the virus of cucumber mosaic can be separated readily from the virus of curly top by taking advantage of the contact period required for passage of the respective viruses through a graft union. Also the virus of ring spot often is separable from the virus of cucumber mosaic in short contact periods. Less often the virus of cucumber mosaic is separated from that of ring spot. It is believed that these separations are due, for the most part, to the difference in tissues in which the viruses are able to travel.

Previous histological investigations of the graft unions of Turkish tobacco have shown that there is at least a meristematic union between stock and cion after 48 hours of contact and that phloem begins to differentiate in the new tissue of the union at least by the seventh day.

Strong circumstantial evidence indicates that parenchyma-inhabiting viruses are able to move from cell to cell in the parenchyma through the plasmodesmata. This would permit the viruses of cucumber mosaic and ring spot to move across the graft union as soon as a parenchyma union became available. The curly-top virus, however, appears to be more or less limited to the phloem, and it is known that it is unable to move through at least certain types of parenchyma. It seems probable, therefore, that the virus of curly top is retained in the cion until differentiation of phloem begins in the graft union.

The apparent greater rate of movement of the ring-spot virus through the graft union as compared with that of the cucumber-mosaic virus might be due to greater mobility resulting from size of particle, rate of increase, or other factors. However, there is a basis for suggesting that this also may be the result of the relation of the respective viruses to tissues. Valleau[2] is of the opinion that the meristematic tissue of tobacco plants is invaded by the ring-spot virus. Apparent occurrence of this virus in pollen and the fact that infected plants recover from severe initial symptoms of the disease support this concept.

Sheffield's[3] work indicates that the virus of aucuba mosaic of tomato does not completely invade the meristematic cells of the growing point of affected plants but progressively invades the cells below the primary meristem as they begin to differentiate into more mature tissues. Some cells are invaded early enough to inhibit development of plastid primordia, others are not. This unequal invasion in which development of plastid primordia is suppressed in early invaded cells and not in later invaded cells, results in the mottled condition of the leaves of affected plants.

If the ring-spot virus is able to invade apical meristem, as Valleau suggests, and if mottling in general results from delayed non-uniform invasion of the cells below the primary meristem of the growing apex of the stems, as Sheffield's results would tend to indicate, it is probable that the ring-spot virus may be able to invade and migrate through cells that are somewhat younger than those that will permit passage of the virus of cucumber mosaic. Hence it is quite possible that the virus of ring spot might pass through graft unions when a meristematic union is available; whereas passage of the virus of cucumber mosaic would be delayed until meristem begins to differentiate into other tissues. In plants grafted to cions carrying both cucumber-mosaic virus and ring-spot virus this might account for the larger number of plants in which only ring-spot virus occurred.

It is true, of course, that some of the plants grafted to cions containing both viruses showed only cucumber mosaic; and it may be argued that in these instances the virus of cucumber mosaic passed the graft union first.

[2] Valleau, W. D. Experimental production of symptoms of so-called recovered ring-spot tobacco plants and its bearing on acquired immunity. Phytopath. **31**: 522–533. 1941.

[3] Sheffield, F. M. L. The development of assimilatory tissue in Solonaceous host plants affected with the aucuba mosaic of tomato. Ann. Appl. Biol. **20**: 57–69. 1933.

This possibility is by no means eliminated, but it is not necessarily true. As was noted earlier, there is evidence indicating that local infection by the strain of ring-spot virus used does not always result in systemic infection. Therefore, even with plants showing only cucumber mosaic, the virus of ring spot may have passed through the graft union first, but failed to establish itself sufficiently to cause systemic infection.

On the basis of information already available on graft transmission of "yellow" and "mosaic" types of viruses it seems reasonable to expect that the mosaic types in general will be found to be transmissible by short contact periods between diseased and healthy tissue. Apparently a parenchymatous union, and perhaps sometimes only a meristematic union, is sufficient to permit virus passage. Such unions might occur between tissues of widely different species of plants. Even though the grafted tissue failed to remain alive for more than a few days it might make a union that would permit passage of virus. If this should prove to be true the graft technique, if extensively tested, might prove to be of considerable value in extending knowledge as to virus host range and in determining virus relationships, in groups of plants such as Rosaceae, with viruses that are not juice transmissible and for which insect vectors are unknown or have limited host ranges.

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CARNATION MOSAIC

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(Accepted for publication January 2, 1943)

A disease of carnations (*Dianthus caryophyllus* L.) with symptoms resembling those of mosaic diseases of other plants, first came to the attention of the writer in the fall of 1939. Since then it has been observed frequently, affecting many of the leading commercial varieties of greenhouse carnations.

Others previously had encountered maladies of carnations quite similar to, if not identical with, the disease referred to here as carnation mosaic. As early as 1912 Peltier (5), working at the University of Illinois, became interested in a disease that he referred to as carnation yellows. He described some leaf symptoms that appear to be very similar to those associated with carnation mosaic, but he did not mention flower breaking. In a limited test, Peltier grafted healthy cions to diseased plants and found that mottling and flecking occurred in the cion shoots. Viruses being little understood at that time, this transmission apparently did not suggest a virus disease to Peltier. Lamkey (4), who followed Peltier in the work at Illinois, reached no conclusion as to the cause of yellows, but in his discussion he suggested that it might possibly be caused by a virus. Fukushi (3) included carnation mosaic in a list of virus diseases found in Japan in 1932, but described no symptoms and cited no evidence that the disease was caused by a virus. In 1937 Smith (7) referred to a disease of carnations found in England as a suspected virus disease, in which the leaves are mottled and the flowers are broken. Smith considered that further investigation was necessary to definitely establish its cause. Asuyama (2), in 1938, reviewing recently reported diseases of cultivated plants in Japan, summarized a paper on mosaic disease of the carnation by Abe (1), another Japanese worker. He briefly described symptoms of leaf mottling and flower breaking closely resembling those associated with carnation mosaic in the United States and referred to the pathogen as a virus, but cited no evidence to prove that the disease actually was caused by a virus. In 1939, Pirone (6) listed carnation mosaic as a virus disease, but gave no evidence that would indicate the cause to be of virus nature.

From these accounts, chiefly mere mentions or imperfect descriptions, one cannot be certain that the various maladies listed are the same or that any one of them is identical with the disease referred to in this paper as carnation mosaic. Also, none of these accounts gives conclusive experimental evidence that any carnation trouble referred to is caused by a virus.

[1] Appreciation is expressed to Dr. Leo R. Tehon for his helpful suggestions given during the course of this study and in the preparation of the manuscript; also, acknowledgement is made to Dr. R. R. Kudo, Department of Zoology, University of Illinois, for translating that portion of Asuyama's article dealing with carnation mosaic.

SYMPTOMS

Plants affected with carnation mosaic show mottling and flecking of leaves and stems and breaking and distortion of flowers; also, the general vigor and productiveness of diseased plants are reduced. Young leaves of affected plants show mottling of light- and dark-green patches or streaks that run parallel with the midribs, causing the affected leaves to appear lighter green than those of healthy plants (Fig. 1). Older leaves of affected

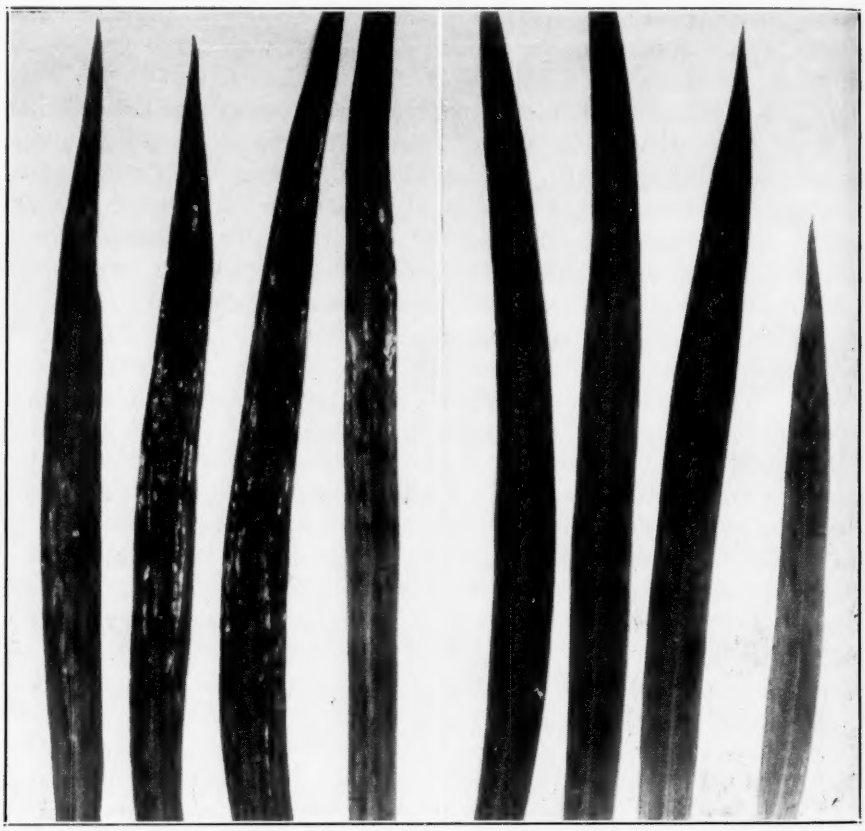

FIG. 1. Carnation mosaic. At the left, leaves in an advanced stage of infection showing mottling, flecking and streaking; at the right, normal leaves. Photographed with transmitted light.

plants show whitish, sunken, elongated flecks or streaks, which eventually can become reddish, purplish or brown. Sometimes severely affected leaves are blighted. Also, stems of diseased plants frequently show whitish flecks or streaks similar to those shown on leaves.

In the colored varieties, flower breaking accompanies mottling and flecking of leaves (Fig. 2). Broken flowers show white or light-colored streaks that parallel the veins of the petals, fanning out from the base toward the tip. In the white-flowered varieties, the typical leaf symptoms appear, but

the flowers do not show breaking because they are white. Regardless of color, flowers of affected plants usually are distorted and of poor quality.

TRANSMISSION STUDIES

Repeated attempts to associate a fungus or bacterium with the sunken necrotic flecks in the older leaves of affected plants by microscopic examination and culture studies have failed, but the infective principle has been successfully transmitted from diseased to healthy plants by grafting and has been carried repeatedly from one crop of plants to the next in cuttings taken

FIG. 2. Carnation mosaic. At the left, an opening flower bud of King Cardinal variety showing broken or white-streaked petals as a result of inoculation by grafting; at the right, a normal flower of the same variety.

from diseased plants. In a preliminary experiment, a total of 9 cions of healthy King Cardinal carnations were grafted to 4 diseased plants of the Pink Treasure variety. All cions have developed branches with typically mottled and flecked leaves, and all that have flowered have produced broken and distorted flowers.

In a more extensive grafting experiment, 315 plants were propagated from carefully selected healthy plants of the King Cardinal variety. To 215 of these healthy plants, cions from diseased plants of the King Cardinal and Pink Treasure varieties were grafted. The remaining 100 plants, consisting of 2 or 3 plants of each clone of healthy plants, to which no cions were grafted, were retained as checks. Out of the first series of 110 grafted plants, 91 successful grafts were obtained, and in the second series of 105 grafted plants, 101 were obtained. Although this experiment still is in

progress, the results obtained thus far are very indicative. In the first series, 90 plants out of 91 show marked symptoms of mottling and flecking of leaves, and of 32 plants that have flowered all yielded typically broken and distorted flowers. In the second series, which was started somewhat later than the first series, 78 plants out of a total of 101 show symptoms of leaf mottling and flecking, and of 20 that have flowered all produced broken flowers. None of the 100 check plants shows symptoms of the disease. The results of these grafting experiments show that the infective principle can be transmitted from stock to cion and from cion to stock by grafting, providing good evidence that the disease is caused by a virus.

Thus far the virus has not been mechanically transmitted to healthy plants. An experiment designed to transmit the virus from diseased to healthy plants by alternate pinching or topping of diseased and healthy plants has given negative results. Likewise, an experiment designed to induce the disease in healthy plants by rubbing the leaves with juice from diseased plants, after the leaves were dusted with carborundum powder, has been unsuccessful.

Observations made in commercial plantings of carnations indicate that disease-free lots of plants can become infected when grown near mosaic plants in the same greenhouse or field plot. Results of an experiment conducted last year gave similar indications. In this experiment, 300 young plants of King Cardinal carnations were purchased from a grower and were separated at random into two lots. One lot of 200 plants was grown in a commercial field plot during the summer, adjacent to rows of other carnations containing a high percentage of mosaic, while the other lot of 100 plants was grown in the greenhouse, where it was isolated from plants known to be diseased. To a considerable extent plants grown in the greenhouse were protected from insects, while no attempt was made to control insects in the field. In the fall plants grown in the field plot were brought to the greenhouse and planted in a bed adjacent to those grown in the greenhouse all summer for comparison. By the following spring 30 per cent of the field-grown plants were mosaicked, showing typical symptoms of leaf mottling and flower breaking, while only 6 per cent of those grown in the greenhouse showed symptoms of the disease. Since all attempts to transmit the virus from diseased to healthy plants by mechanical means have failed, it is strongly suspected that insects are vectors. Insects as vectors would, of course, explain the rapid spread of the virus from infected to healthy plants in commercial plantings.

SUMMARY

Carnation mosaic has been observed frequently in Illinois greenhouses and similar, if not identical, carnation troubles have been reported previously from the United States, Europe, and Asia.

Leaves and stems of affected plants are mottled, streaked and flecked and flowers of colored varieties are broken.

Results of experiments and observations indicate that carnation mosaic is caused by a virus, evidence which earlier workers have not presented. The virus can be transmitted readily from diseased to healthy plants by grafting, it can be carried from one crop to the next in cuttings taken from infected plants for propagation, and in the field it is spread from diseased to healthy plants, possibly by insects.

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DIPLODIA PINEA AND SPHAEROPSIS MALORUM ON SOFT PINES

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(Accepted for publication March 19, 1943)

Occasional reports have been made of the occurrence of species of *Sphaeropsis* on cankers or dead areas on trunks or branches of eastern white pine (*Pinus strobus* L.), Himalayan pine (*P. griffithii* McClell.), and limber pine (*P. flexilis* James). In some cases the organism has been reported as *Sphaeropsis ellisii* Sacc. (3, 4), which has large brown continuous, or rarely one-septate, spores produced in pycnidia. The fruiting bodies and spores correspond to those of *Diplodia pinea* (Desm.) Kickx of which *S. ellisii* is a synonym (13). This fungus also has been reported by Slagg and Wright (11) as the cause of the death of seedlings of Colorado piñon pine (*P. cembroides* var. *edulis* (Engelm.) Voss). Another species of *Sphaeropsis* with spores smaller than those of *D. pinea* and more closely resembling *S. malorum* Pk., the pycnidial stage of *Physalospora obtusa* (Schw.) Cke., has been found on dead areas on branches or trunks of eastern white pine. Very little is known regarding the conditions under which these two fungi infect soft pines. In connection with a study of *D. pinea* on hard pines (13) a few observations on the occurrence of these fungi and a series of inoculations were made.

DIPLODIA PINEA

Cankers caused by *Diplodia pinea* have been noted by the writer, particularly on eastern white pines growing in unfavorable locations, on poor soil, or densely shaded, or weakened by environmental agencies, or injured by mechanical means or by pruning. Crandall (2), however, found that *D. pinea* was the cause of a collar and root rot of white pine seedlings; and Pirone (9) obtained positive results from the inoculation of the stem of a 5-year-old white-pine seedling with mycelium from a single-spore culture of *D. pinea*. A report by Waterman and Miller (14) of a tip blight of a white pine growing in the vicinity of Austrian pines and Douglas firs heavily infected with *D. pinea* also indicates that under certain conditions the fungus is parasitic on eastern white pines. The parasitism of the fungus on Colorado piñon pine is evident from the investigations of Slagg and Wright (11).

In the study of *Diplodia pinea* on hard pines (13) three vigorously growing white pine trees in an experimental plot were inoculated by the writer with mycelium of the fungus from single-spore cultures isolated from infected needles of Austrian pine. The results were negative in all cases. Six inoculations on other white pines in the same plot were made with mycelium from single-spore cultures of *D. pinea* isolated from pycnidia on a canker around an old pruning wound on a white pine tree in Rhode Island. Two inoculations each were made by applying the inoculum in the following

ways: near the base of uninjured leaves of the new growth when the leaves were about one inch long; on leaf scars from which the newly developed leaves had just been removed; in wounds on twigs of the new growth made by cutting with a sterilized scalpel a small triangular section of bark under which the inoculum was inserted. After the inoculum was applied, the portion inoculated was wrapped with moist cotton and then covered with a strip of waxed paper firmly tied in place. The coverings were removed about 10 days after inoculation. Controls were made in the same way without the introduction of the inoculum. No positive results were obtained in any of the inoculations.

SPHAEROPSIS MALORUM

In 1885 Peck (8, p. 98) reported a fungus on *Pinus strobus* as a new variety of *Diplodia pinea*, namely, *D. pinea corticola*. This variety is considered by Stevens (12) to be a synonym of *Sphaeropsis malorum*. His examination of Peck's specimen showed that the spores measured 22–24 μ × 11–13 μ, smaller than those of *D. pinea*, but within the range of *S. malorum*. Hesler (6) listed *P. strobus* as a host of *S. malorum* as indicated by collections only. The results of his cross inoculations of *P. strobus* with *S. malorum* isolated from apple were negative (5). Hedgcock (4) reported *S. ellisii* on *P. griffithii* (*P. excelsa* Wall.) from Pennsylvania. Notes by Dearness, accompanying the collection, indicate that the spores measured 20–25 μ × 7–8 μ, which he considered the same as those of *Macrophoma pinea* Passer. This latter fungus is described as having hyaline one-celled spores measuring 22.5 μ × 7.5 μ (10, p. 198). In an examination of the specimen by the writer a very few brown unicellular spores were found with spore measurements as given by Dearness and corresponding with spores of *S. malorum*. The occurrence of *S. malorum* causing a disease of Himalayan pine in Pennsylvania following winter injury was reported by York (15) and Jump (7). Cross inoculations proved that the fungus would infect through wounds made on the twigs of young apple trees and of 3-year-old white pines. Inoculations on 2- and 3-needle hard pines gave negative results.

In the present study a series of inoculations was made with *S. malorum* spores produced from single-ascospore cultures of *Physalospora obtusa* isolated by Ayers (1) from apple twigs in Massachusetts. Young healthy trees of *Pinus strobus* and of the hard pines, *P. nigra* Arnold, *P. sylvestris* L., and *P. resinosa* Ait., growing in the experimental plot, were inoculated with crushed pycnidia containing mature spores. The methods of applying the inoculum to the parts of the trees indicated in table 1 were the same as in the inoculations of *P. strobus* with *Diplodia pinea* mentioned above. From the thirty inoculations the one positive infection occurred on *P. sylvestris* around the base of a dead twig stub immediately adjacent to and partially included in the wound into which the inoculum was inserted. No canker formed on the living branch and the fungus was reisolated only from the

base of the dead twig stub. In all other cases the inoculated wounds healed over, as did those of the controls.

TABLE 1.—*Results of inoculations of species of Pinus with Physalospora obtusa isolated from apple twigs*

Part inoculated	Number of inoculations and infections on species of *Pinus*							
	nigra		*sylvestris*		*strobus*		*resinosa*	
	Inoculations	Infections	Inoculations	Infections	Inoculations	Infections	Inoculations	Infections
Buds uninjured	3	0	3	0	0	0	2	0
Leaves uninjured	1	0	1	0	1	0	2	0
Leaf scars	1	0	1	0	1	0	1	0
Twig wounded	2	0	4	1	3	0	4	0

SUMMARY

The results of these observations and inoculations indicate that neither *Diplodia pinea* nor *Sphaeropsis malorum* is parasitic upon the leaves and twigs of the new growth on young vigorously growing white pines. Both fungi, however, occasionally may be contributing factors in the unhealthy condition of soft pines that have been weakened primarily by other agencies. Improved growing conditions and the prevention or protection of wounds will lessen materially the possibility of infection.

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SPHAEROTHECA HUMULI VAR. FULIGINEA ON DELPHINIUM IN CALIFORNIA

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(Accepted for publication March 17, 1943)

The presence in California of *Sphaerotheca humuli* (DC.) Burr. var. *fuliginea* (Schlecht.) Salmon on *Delphinium* spp.,[1] in addition to the ubiquitous *Erysiphe polygoni* DC., is potentially significant in a breeding program (3) for resistance to this disease.

Several plants of *Delphinium amabile* Tidestr. in the university glasshouses, West Los Angeles, were observed in February, 1942, to have many perithecia of the powdery mildew, *Sphaerotheca humuli* var. *fuliginea*, which may have been brought in on various transplanted native *Delphinium* spp. present at the time. Although no published record of this fungus on *Delphinium* in North America has been found, specimens examined in the University of California herbarium at Berkeley showed the following collection of this fungus under the label *S. humuli:* Berkeley, Alameda Co., Calif., on cultivated *D. consolida* L., July, 1924 (H. E. Parks 2759); Alton, Humboldt Co., Calif., on *D. trolliifolium* Gray, April 24, 1927 (J. P. Tracy 7950); Middle Yager Creek, Humboldt Co., Calif., on *D. trolliifolium,* July 6, 1930 (J. P. Tracy 8857); Coyote Peak, Bald Hills, Humboldt Co., Calif., on *D. trolliifolium*, August 6, 1933 (J. P. Tracy 12959). The Berkeley collection was made in the university botanical garden and carried the notation, "Destructive on this host"[2]; since this area has been rather well botanized it is surprising that there are no known later collections of the parasite there. Mr. J. P. Tracy stated[3] that the mildew ". . . on the wild *Delphinium trolliifolium* is frequent and often damages the host severely" in Humboldt County; he had no record of it on other wild species there. The Plant Disease Survey[4] had no additional records of this mildew on delphinium.

It is evident that the pathogen has been established on native delphiniums in northwestern California for at least 16 years, and was present in the state even earlier. Since other specimens (J. P. Tracy 8886, 9672, 13077½, and H. E. Parks 5102) on cultivated perennial delphinium and rocket larkspur from Humboldt County proved to be *Erysiphe polygoni,* it is uncertain whether the second mildew is established on cultivated varieties in that region.

Study of the various specimens showed several characteristics that allow rapid identification of *Sphaerotheca humuli* var. *fuliginea* under a dissecting microscope of 72× magnification, using lateral illumination. The cells

[1] Several specimens were kindly made available for study by L. Bonar and G. A. L. Mehlquist of the University of California, and by J. P. Tracy, Eureka, California.

[2] H. E. Parks, in correspondence dated February 6, 1943, stated "The fine leaves were well covered with perithecia. . . ." Apparently exsiccati of this collection are fragmentary and relatively rare. Identification of the host was confirmed by G. A. L. Mehlquist.

[3] Letter dated January 30, 1943.

[4] Letter from J. A. Stevenson, dated February 6, 1943.

of the perithecial wall are large and decidedly convex, with the cell sutures rather deeply indented. By contrast, the walls of *Erysiphe polygoni* are composed of smaller cells which are relatively smooth or occasionally concave (*i.e.*, with the central portion of the cell collapsed, a condition apparently most common on immature perithecia). The walls of *E. polygoni* are also darker than those of *S. humuli* var. *fuliginea* and are usually more refractive. In all of the material examined the perithecia of *E. polygoni* were more or less embedded in aerial mycelium and conidiophores, but the other mildew had scant mycelial development; this agrees with the condition described by Blumer (1) for *S. delphinii* Karsten. The conidiophores projecting from the folded margins of leaves, examined as above, showed that conidia of *E. polygoni* were borne singly whereas those of *S. humuli* var. *fuliginea* were borne in chains, a distinction well illustrated by figures 2b and 2d of Brodie and Neufeld (2).

Examination under the compound microscope of many samples of perithecia from a large number of plants of the *Delphinium elatum* group (collected Aug. 10, 1942, in a field planting in West Los Angeles), indicated that the mildew was entirely *Erysiphe polygoni*. After the external structural differences of the perithecia were appreciated, reexamination of the material under the dissecting microscope disclosed small colonies of *Sphaerotheca humili* var. *fuliginea*, as well. Identification was confirmed in all cases by examination under high magnification. Because the new parasite is not so vigorous in its development as *E. polygoni*, it was overlooked by the usual methods of examination. This collection demonstrated that the fungus can become established under outdoor conditions in Southern California.

The new pathogen was found to have perithecia 60–89 μ (average 76.3 μ) in diameter, with conspicuous wall cells 13–28 μ (average 21.3 μ) in width. The asci were 46–88 × 43–71 μ (average 63.7 × 56.8 μ) and the eight ascospores were 13–26 × 13–18 μ (average 18.1 × 14.5 μ). The perithecial diameter is larger than that given in Salmon's monograph (5) for *Sphaerotheca humuli* var. *fuliginea* but within the limits of specimens later (6) referred to this variety; the measurement likewise fits *S. fuliginea* (Schlecht.) Salmon (*sic*) of Blumer (1) but is larger than *S. delphinii* which he separates from it. The size and conspicuousness of the perithecial wall-cells, the recognized criterion of separation of *S. humuli* and its variety *fuliginea*, place the fungus in the latter category, although the size is somewhat less than that reported by Blumer (1) or Salmon (5). The size of the asci and ascospores is not sufficiently distinct to aid in separation of the two fungi.

There apparently are no reports of *Sphaerotheca humuli* on Ranunculaceae but the variety *fuliginea* has been reported on *Delphinium grandiflorum* L. (1) in Siberia, *Paeonia anomala* L. (1) in Russia, *Thalictrum alpinum* L. (1, 5) in Norway and Sweden, *T. minus* L. (1) and *T. simplex* L. (1, 5) in Siberia, and *Trollius europaeus* L. (1) in Italy. *Caltha palustris* L., listed as a possible host in Salmon's monograph (5), was later (6) deleted.

Thalictrum angustifolium L., listed by Oudemans (4), is obviously an error, since the sources cited give no such host.

It is possible that *Sphaerotheca humuli* var. *fuliginea* is established on delphiniums in areas other than California without having been reported. The tolerance of *Erysiphe polygoni* to low humidity (2, 7) may explain the fact that it is more abundant than *S. humuli* var. *fuliginea* on delphiniums in California. However, the new mildew might be damaging in more humid sections.

SUMMARY

Sphaerotheca humuli var. *fuliginea* has been present on cultivated and wild *Delphinium* spp. in California for at least 19 years. No published records have been found of the occurrence of this fungus on *Delphinium* or other Ranunculaceae in North America, but it has been reported elsewhere on several representatives of the family, mostly in northern Europe and Siberia.

Although this parasite apparently is rather rare on present commercial delphiniums it is a potential threat to new types which may be developed. The determination of the present distribution of the fungus is, therefore, of interest. This is facilitated by a method for rapid examination of quantities of mildew material to determine whether *Sphaerotheca humuli* var. *fuliginea* is present with the ubiquitous *Erysiphe polygoni*.

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PHYTOPATHOLOGICAL NOTE

Mercury Compounds Applied to Banana Plants in the Field.[1]—Mercury compounds kill *Fusarium oxysporum cubense*[2] and, therefore, prevent its spread[3] in low concentrations in the soil. A series of studies was made in the field to determine the effect of the mercury compounds on the banana plant and on the Panama-disease organism. In these experiments the following mercury compounds were used: DuBay 1155–HH, 5 per cent ethyl mercury iodide (Bayer-Semesan Co.); Hortosan Potato Dip, 8 per cent nitrophenol-mercurihydroxide and chlorophenolmercurihydroxide (Imperial Chemical Industries Ltd.); mercurous chloride; and mercuric chloride.

In the first experiment 3 banana plants were selected and 2 plots $2' \times 2'$ dug 2′ from each plant, close together. The soil from each plot to a depth of 6 inches was removed to a canvas. Hortosan was mixed with the soil of one plot from each tree at the rate of 2 oz., 4 oz., and 8 oz., per sq. ft., respectively, and replaced. The nontreated soil was replaced on the check plots. At the end of 1 month the roots at the edges of the 8-oz. plot had turned up and were hypertrophied. At the end of 10 weeks the soil was again removed from all the plots and the roots separated and weighed. The roots from the Hortosan 2-oz., 4-oz., and 8-oz. plots weighed 14 g., 8 g., and 0 g. respectively, while the checks weighed 38 g., 28 g., and 59 g. The soil was then replaced. Five months later the roots from the Hortosan plots totalled 83.4 g. and the checks 79.7 g. No root injury was observed. Five months after the beginning of this experiment the soil was tested for the growth of *Fusarium oxysporum cubense*. Tubes were half-filled with soil from the treated and nontreated areas, autoclaved, planted with the fungus, and the growth rate compared.[4] The soil from the Hortosan plots permitted no growth, while the checks produced normal growth. At 7 months the same results were obtained. After the Hortosan had weathered in the soil for 9 months the *Fusarium* growth readings were 1.5 cm., 0.0 cm., and 0.0 cm., with the checks normal at 3.1 cm., 3.1 cm., and 4.0 cm. After 11 months the 2-oz. per sq. ft. treatment permitted a normal growth of the Panama-disease fungus, but the 4-oz. and 8-oz. treatments gave reduced growth.

In the second experiment, at the time of planting, the mercury compounds were mixed with the soil around the plant within a radius of 2 feet. The application of 2 oz. per stool of Hortosan or 1 oz. of DuBay, mercurous chloride, or mercuric chloride at the time of planting apparently gave no injury. A 1-oz. per plant treatment of Hortosan inhibited the growth of

[1] Acknowledgment is due the Jamaica Banana Producers' Association for support in this research.

[2] Meredith, C. H. The effect of chemicals on *Fusarium oxysporum cubense* growing in the soil. Phytopath. **32**: 182–184. 1942.

[3] Meredith, C. H. The effect of soil and chemical mixtures on the growth of *Fusarium oxysporum cubense*. Phytopath. (in the press).

[4] Meredith, C. H. The growth of *Fusarium oxysporum cubense* in the soil. Phytopath. **31**: 91–93. 1941.

Fusarium oxysporum cubense for 3 months on acid soil and 2 months on neutral soil. In plots on 2 different soils the 2-oz. treatment of Hortosan has given a greater growth of the banana plant than the growth on the non-treated soil.

In the third experiment Hortosan potato dip was spread over the area covered by the roots of 3 different plots. There were 2 stools in each plot. The Hortosan was applied at the rate of 3 oz. per 100 sq. ft., 6 oz. per 100 sq. ft., and 12 oz. per 100 sq. ft. Soil samples were taken and growth readings made with *Fusarium oxysporum cubense*. The readings were not consistent, perhaps due to the fact that the mercury compound was sprinkled on the surface rather than mixed with the soil, as in the other experiments. There was no injury to the banana plants observed for 8 months after this treatment.

The banana plant apparently can withstand higher concentrations of mercury compounds than *Fusarium oxysporum cubense*. The Hortosan potato dip was observed to stop the growth of the Panama-disease organism in the soil after 9 months weathering. The Hortosan was more effective in acid soils than in neutral.—CLIFFORD H. MEREDITH, Glenleigh Laboratory, Highgate P. O., Jamaica, B. W. I.